IONIC REGULATION IN GOLDFISH, *CARASSIUS AURATUS*, ACCLIMATED TO CONSTANT AND DIURNALLY-CYCLING TEMPERATURE CONDITIONS

by

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ABSTRACT

The effects of a diurnal sine-wave temperature cycle ($25^{\circ} \pm 5^{\circ}$ C) on the water-electrolyte status of goldfish, *Carassius auratus*, was assessed through determination of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-} and water content in plasma, Red blood cells and muscle tissue. Animals were also acclimated to static temperatures (20°C, 25°C, 30°C) corresponding to the high, low and mid-point temperatures of the cycle. All groups were sampled at 03:00, 09:00, 15:00 and 21:00 hr. Hemoglobin content and packed cell volume, as well as electrolyte and water levels were determined for each animal and red cell ion concentrations and ion : hemoglobin ratios estimated.

Cycled animals were distinct from those at constant temperatures in several respects. Hematological parameters were elevated above those of animals at constant temperature and were, on a diurnal basis, more stable. Red blood cell electrolyte levels varied in an adaptively appropriate fashion to cycle temperatures. This was not the case in the constant temperature groups. Under the cycling regime, plasma ion levels were more diurnally stable than those of constant temperature fish. Although muscle parameters in cycled fish exhibited more fluctuation than was observed in plasma, these also tended to be relatively more stable than was the case at constant temperature.

Erythrocytic data are discussed in terms of their effects on hemoglobin-oxygen affinity while plasma and muscle observations were considered from the standpoint of overall water-electrolyte balance.

In general, cycled fish appeared to be capable of stabilizing overall body fluid composition, while simultaneously effecting adaptively-appropriate modifications in the erythrocytic ionic microenvironment of hemoglobin. The sometimes marked diurnal variability of water-electrolyte status in animals held at constant temperature as opposed to the conservation of cycled fish suggests that this species is, in some fashion, programmed for regulation in a thermally-fluctuating environment. If this interpretation is valid and a phenomenon of general occurrence, some earlier studies involving constant acclimation of eurythermal species normally occupying habitats which vary in temperature on a daily basis may require reconsideration.

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INTRODUCTION

Aquatic habitats in the north temperate zone commonly exhibit substantial seasonal temperature variation. Shallow slow-flowing waters may, in addition, undergo marked fluctuations on a diurnal basis as well. Moreover, in the Great Lakes region, as in many other areas of Canada, what might be termed 'natural' thermal regimes, are increasingly subject to alteration as a consequence of heat discharges from a variety of industrial and municipal activities. Commonly, these take the form of localized modification of seasonal baseline temperatures, exaggeration of daily temperature variations and, episodically, abrupt increases or decreases in temperature as heated effluent discharges are initiated or interrupted. Because of these considerations, the thermal biology of aquatic organisms, and particularly fishes, has been increasingly emphasized during the past decade.

Temperature is without question the principal controlling factor[1] in the aquatic environment and influences organismic responses to virtually all other limiting, accessory, directing and other agencies. Among the most obvious

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1. Throughout this thesis, the term 'factor' is used in the sense employed by F.E.J. Fry (1947), i.e., as implying an effector agency rather than environmental identity.

effect of temperature is its influence upon oxidative metabolism. Not uncommonly the oxygen requirements of fishes, for example, change by an order of magnitude, or more, over the tolerated range of temperatures. Under such circumstances, however, oxygen solubility in water is altered in a manner which is highly inappropriate; the teleost confronted with an increase in environmental temperature must satisfy substantially increased oxygen requirements under circumstances of reduced oxygen availability. If the animal is unable to utilize behavioural means to avoid thermal stress, responses are normally seen at both the branchial exchanger and blood gas transport system levels. In the former category are included adjustments in ventilatory flow, cardiac output (i.e. branchial perfusion) and effective gill surface area. All are employed. All however, involve increased metabolic costs, are inherently self-limiting and/or prompt secondary stresses which require some form of compensatory response. Notable among the latter are the perturbations in water-electrolyte and acid-base balance occasioned by increased endosmosis, electrolyte losses stemming from renal responses to overhydration and enhanced passive ion efflux from the gills and buccopharyngeal epithelium.

Adjustments in the blood gas transport are seen at several levels. Although inconsistent in occurrence, increases in erythrocyte numbers and overall hemoglobin concentration, coupled with reductions in red cell volume have been reported. In species possessing polymorphic hemoglobin systems, variations in the relative and/or absolute abundancies of specific hemoglobin types are commonly seen. In some instances, at least, it is apparent that hemoglobins possessing transport characteristics appropriate to particular temperature conditions are formed. At another level, hemoglobin-oxygen affinity can be modulated by a variety of factors, always given, of course, that the specific hemoglobins in question are modulator sensitive. Since most, though by no means all hemoglobin oxygen interactions are exothermic in nature, increases in temperature normally prompt reductions in affinity and facilitate oxygen release at the tissue level. Similarly, the decreases in pH which accompany increases in temperature would be expected to reduce hemoglobin-oxygen affinity in hemoglobins characterized by Bohr effects. A variety of organophosphate modulators, notably ATP and GTP in fishes, can effect affinity if their intraerythrocytic concentrations alter with temperature. Finally, during the past decade it has become increasingly apparent that modulation of affinity can also be effected by inorganic ions. Chloride, for example, mimics organophosphates in this respect. The action of magnesium is less direct. Magnesium ions complex with ATP and GTP. Since the complex does not interact with hemoglobin the effect of increasing magnesium content is essentially one of reducing organophosphate availability for modulatory interaction with hemoglobin.

In earlier studies (Houston & Smeda, 1979; Houston & Mearow, 1979; unpublished observations) it was shown that increases in the acclimation temperatures of the eurythermal carp, Cyprinus carpio and goldfish, Carassius auratus and the more stenothermal rainbow trout, Salmo gairdneri, were associated with adaptively-appropriate modifications in red cell ionic composition. In each instance, chloride levels tended to increase, while those of magnesium declined. Such changes would be expected to reduce the oxygen affinities of modulator sensitive hemoglobins, facilitating oxygen release at the microcirculatory level and assisting in the satisfaction of temperature-induced increases in oxygen requirements. These studies were, however, conducted at constant temperature - a circumstance rarely encountered under natural circumstances. The present investigation was, therefore, undertaken in order to examine possible alterations in erythrocytic ion composition under more ecologically-realistic conditions, and specifically under circumstances in which the animal is exposed to diurnally-cycling temperatures. The species employed, the common goldfish (Carassius auratus) was selected for its general eurythermality and ability to tolerate both abrupt and gradual alterations in environmental temperature. Populations were acclimated for several weeks prior to sampling under a 12 light : 12 dark photoperiod regime to a sinusoidal pattern of daily temperature variations characterized by a mid-point of 25° C, and extremes of 20° and 30° C. Additional groups were maintained at constant temperatures of 20°, 25° and 30° C. Because of the possibility of diurnal variations in ionic composition, all groups were sampled at intervals coincident with the maximum, minimum and mid-point temperatures of the cycled animals, i.e., 03:00, 09:00, 15:00 and 21:00 hours. Chloride, sodium, potassium, calcium and magnesium determinations were carried out on plasma and packed red cell samples, as were estimates of plasma and erythrocytic water content. These values, with whole blood hematocrit and hemoglobin content, permitted calculation of red cell ion concentrations and hemoglobin:ion ratios under the experimental conditions imposed. In addition, because little is known regarding the effects of cycling temperatures upon general ionoregulatory capacity, similar analyses were conducted on muscle as well.

REVIEW OF LITERATURE

A vast literature exists with respect to thermal biology in fishes. Because of this, the following review of literature has been focussed upon selected topics particularly pertinent to the area of investigation. The following have been included:

(1) The thermal biology of the goldfish with particular emphasis upon thermal tolerance and temperature-related variations in oxygen requirement.

(2) Systemic responses to increased oxygen demand.

(3) Hematological responses to increased oxygen demand.

(4) Hemoglobin polymorphisms and the modulation of hemoglobin-oxygen affinity by temperature, pH, organophosphates and inorganic ions.

(5) General features of ionic regulation in freshwater fishes.

(6) The nature of thermoacclimatory electrolyte responses in freshwater fishes.

(7) Cycling temperature studies.

(1) Thermal Biology of the Goldfish

(A) Thermal Tolerance

The thermal tolerance of the goldfish (*Carassius auratus*) has been the subject of a number of studies, the results of which have been summarized in Table 1 and Figure 1. Although

Figure 1. Summary of thermal relationships in the goldfish, *Carassius auratus*. X-Y axis : thermal tolerance utilizing data from Fry, *et al*. (1942) (○) Fry, *et al*. (1946) (▲) and Weatherley, (1970) (●) X-Z axis : Standard oxygen consumption utilizing data from Beamish and Mookherjii (1964) (■) X-Z_2 axis : Thermal Preference utilizing data from Fry (1964) (△)

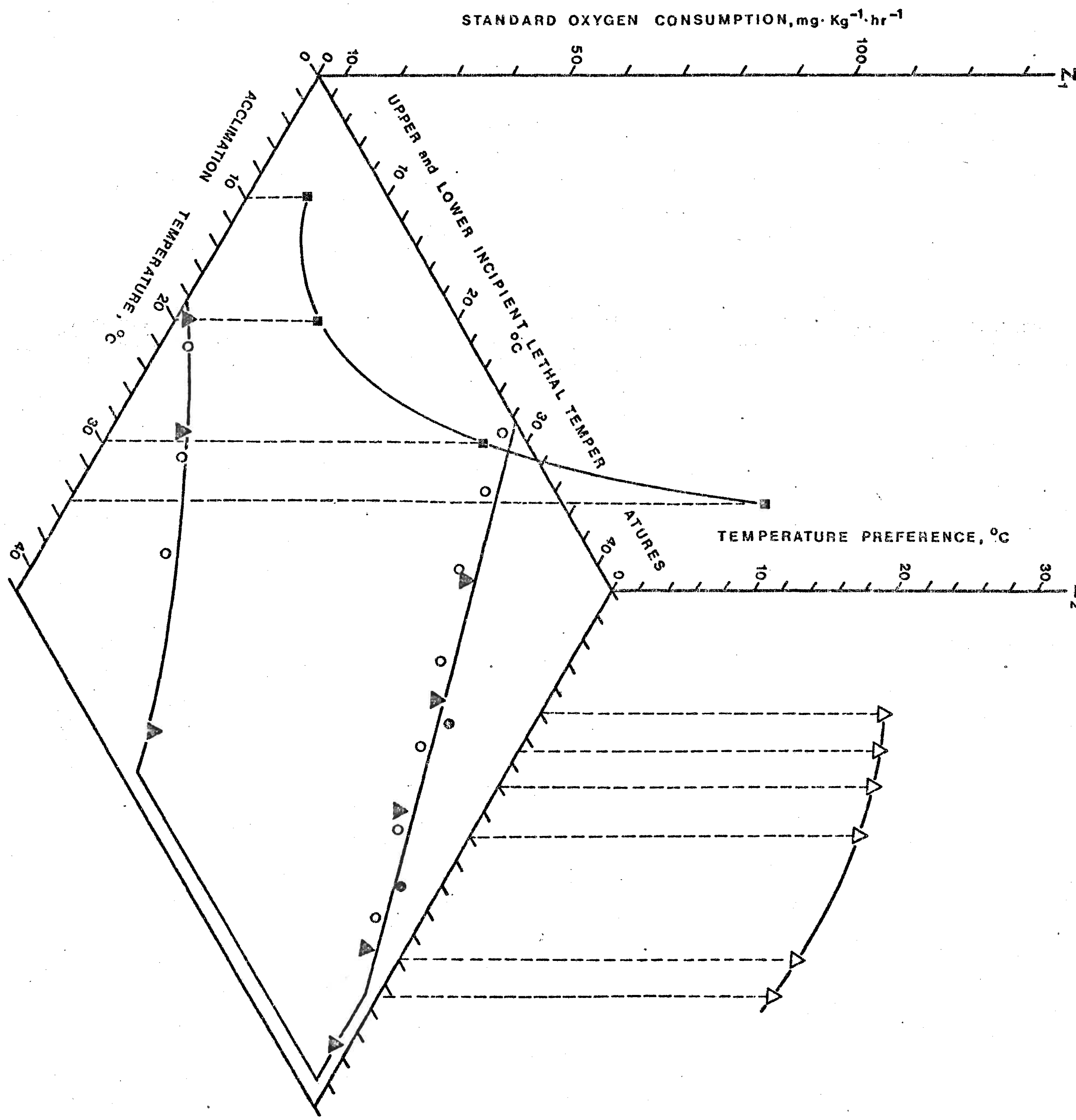

STANDARD OXYGEN CONSUMPTION, mg·Kg⁻¹·hr⁻¹
0
10
50
100
Z1
ACCLIMATION TEMPERATURE, °C
10
20
30
40
UPPER and LOWER INCIPIENT LETHAL TEMPERATURES °C
10
20
30
40
TEMPERATURE PREFERENCE, °C
10
20
30
Z2

TABLE 1 : Upper and lower incipient lethal temperature and/or critical thermal maxima and minima, final thermal preferenda and standard oxygen consumption in thermally-acclimated goldfish, *Carassius auratus*.

(Adapted from Houston, 1980)

STAGE	ACCLIMATION TEMP. °C	OTHER EXP'L CONDITIONS	UPPER LD_{50} or CTmax. °C	LOWER LD_{50} or CT min. °C	ULTIMATE UPPER/ LOWER °C	PREFERENDUM °C	STND O_2 mg/kg·hr	REFERENCES
Juvenile	1-2 5 10 15 20 25 30		28 29.0 30.8 32.8 34.8 36.6 38.6	 2.0 6.0 9.0	41.0/-			Fry, Brett & Clawson(1942)
Juvenile	10 17 19 24 32 38		31 34 - 36.0 39.2 41.0	 1.0 5.0 - 15.5				Fry, Hart & Walker (1946)
	10 12 13 15 16 18 20 22 25 30					24.0 24.7 25.2 26.0 26.5 27.2 27.5 27.9 28.0 28.0		Fry (1964)
Juvenile	17 27	3 atmos O_2 1 atmos O_2 0.5 atmos O_2 3.8 atmos O_2 1 atmos O_2 0.5 atmos O_2	37.1 36.3 35.6 40.0 39.2 38.7					Weatherley (1970)
Juvenile						28.1		Fry (1947)
Juvenile						30		Roy & Johansen (1970)
Adult		Spring Summer Autumn Winter				25.3 27.0 24.0 24.2		Reutter & Herdendorf(1974)
Adult	10 20 30 35						15.7 30.1 72.0 127.0	Beamish & Mookherjii (1964)

a thermal range of 1.0° to 41.0°C is exhibited by this species, several environmental factors are known to influence tolerance at both thermal extremes. These include acclimation temperature, oxygen abundance and photoperiod.

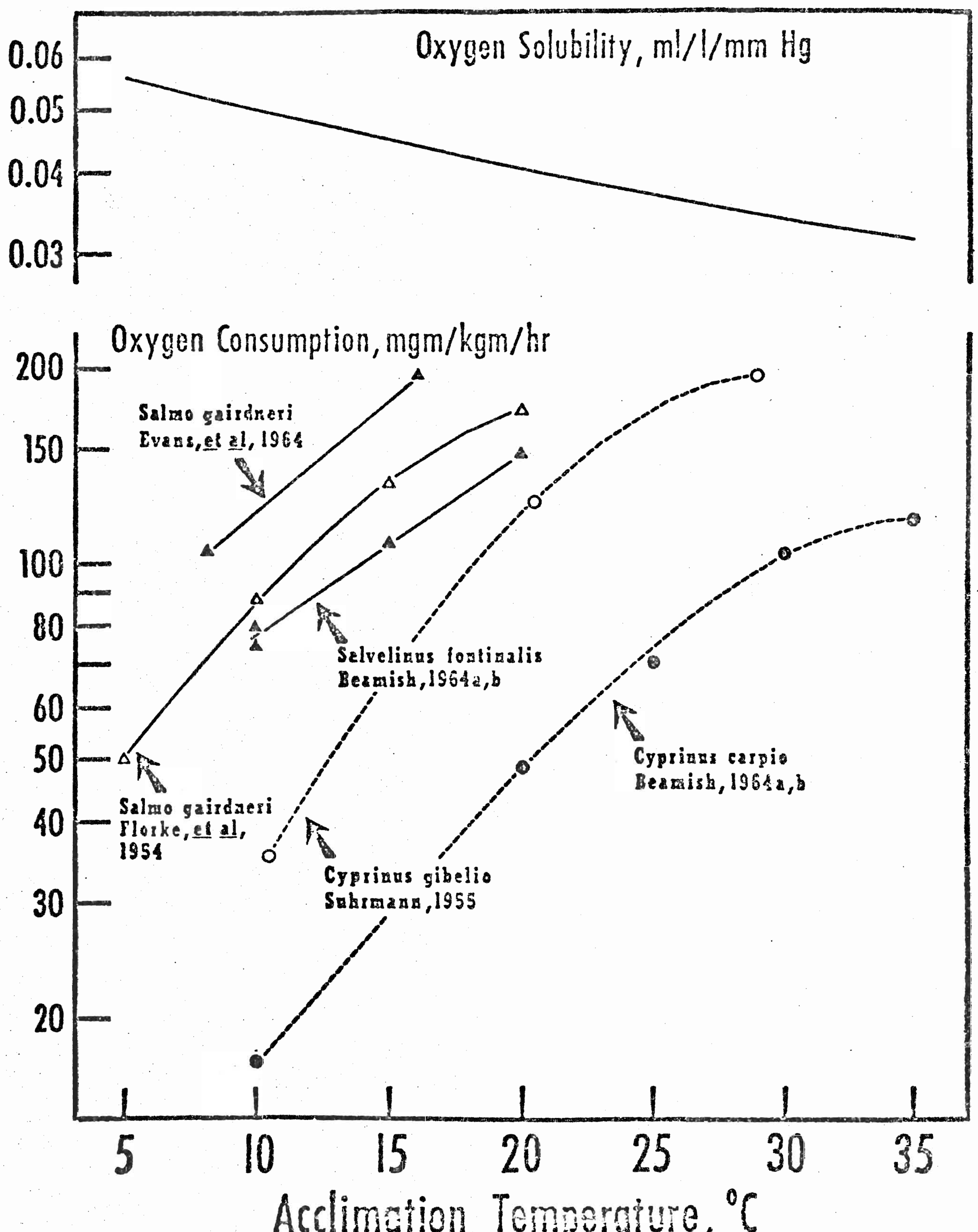
As acclimation temperature increases both the upper and lower incipient lethal temperatures and the preferred temperature increase. Fry *et al*. (1942) reported that with a 3.0°C rise in acclimation temperature, the upper and lower lethal temperatures increase by approximately 1°C and 2°C respectively. Preferred temperatures increase from 24.0°C to 27.9°C over the range of 10.0°C to 22.0°C and then level off at 28.0°C at higher temperatures.

Weatherley (1970) has shown that hyperoxic conditions increase the upper lethal temperature on exposures up to 5 atms. However, tensions in excess of 5 atmospheres convey no further increase.

Appropriate seasonal variations in the temperature tolerance of goldfish were reported by Hoar (1955). Diet, and specifically the type and quantity of fat (Hoar & Dorchester, 1949; Hoar & Cottle, 1952) effect heat and cold resistance, but no obvious relationship between tolerance and fat melting point was demonstrated. Size and sex had no obvious bearing on dietary fat influence. Photoperiodic effects on thermal resistance were demonstrated by Hoar and Robertson (1959) and Weatherley (1973). In the former study, a long photoperiod

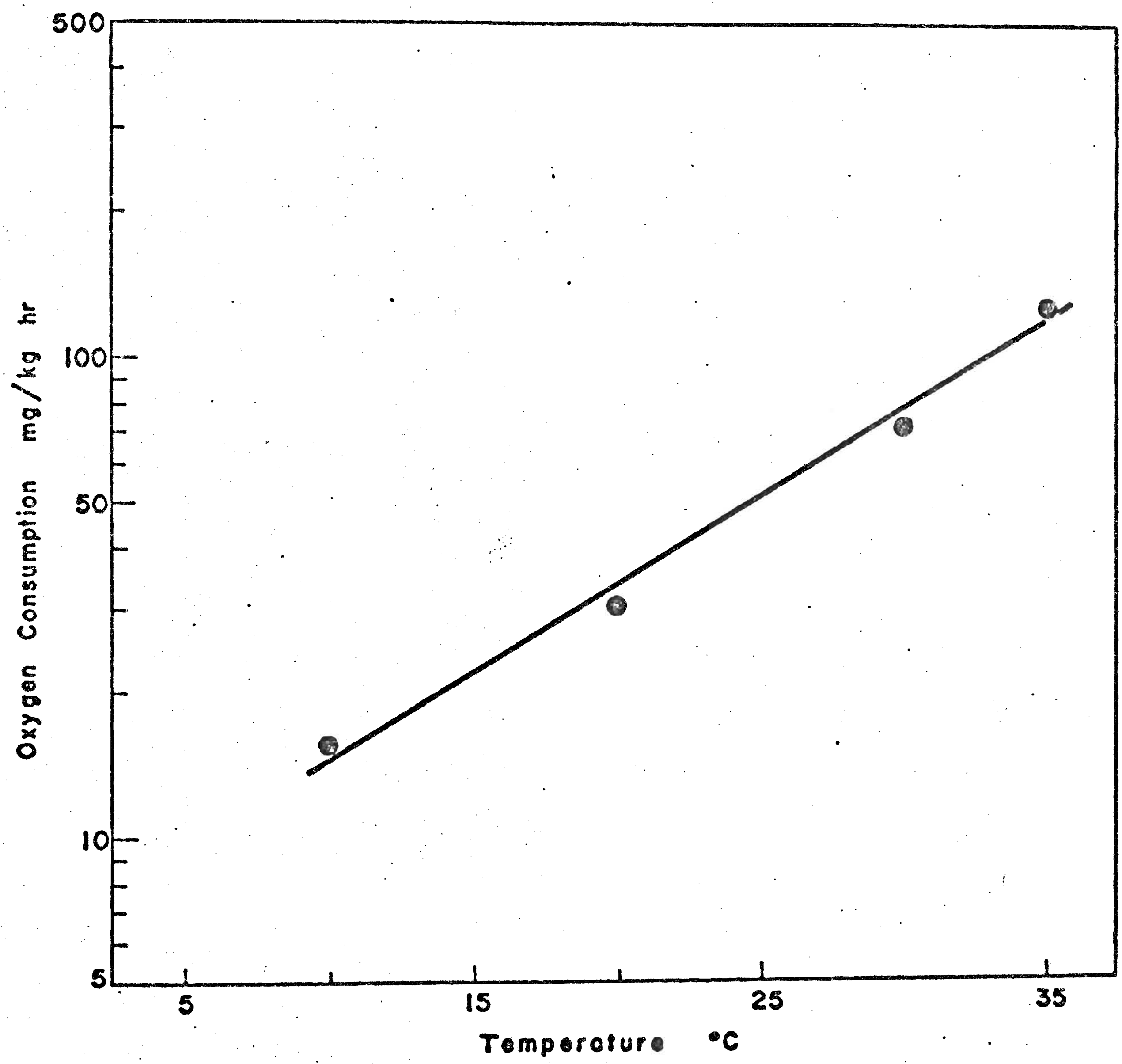
Figure 2 : Changes in oxygen availability and oxygen consumption with temperature in representative cyprinid and salmonid species following thermal acclimation

(taken with permission from Houston, 1973)



Oxygen Solubility, ml/l/mm Hg
0.06
0.05
0.04
0.03
Oxygen Consumption, mgm/kgm/hr
200
150
100
80
60
50
40
30
20
Salmo gairdneri
Evans, et al, 1964
Salvelinus fontinalis
Beamish, 1964a,b
Salmo gairdneri
Florke, et al, 1954
Cyprinus gibelio
Suhrmann, 1955
Cyprinus carpio
Beamish, 1964a,b
5
10
15
20
25
30
35
Acclimation Temperature, °C

Figure 3 : Relationship between standard oxygen consumption and temperature in the goldfish, <u>Carassius auratus</u> (Adapted from Beamish and Mookherjii, 1964).


500
100
50
10
5
Oxygen Consumption mg/kg hr
5
15
25
35
Temperature °C

(16 hr) resulted in increased resistance to sudden increases in temperature, whereas short photoperiod (8 hr), prompted some increase in chilling resistance. There was some evidence of increased thyroid activity in 8 hr fish and it was suggested that this may have been related to enhanced cold resistance. Weatherley (1973) showed that constant illumination for 11 to 17 days resulted in prolonged survival times at upper lethal temperatures for goldfish acclimated to a range of temperatures. The interaction of extended light exposure and hyperoxia appeared to be synergistic in nature.

Values for final preferendum in the literature range from 24.0°C (Reutter & Herdendorf, 1974) to 30.0°C (Roy & Johansen, 1970). Appropriate seasonal changes in this parameter were noted by Reutter and Herdendorf (1974).

B) Temperature-Related Variations in Oxygen Requirements

Increases in environmental temperature constitute a uniquely stressful challenge to teleosts, for increased metabolic oxygen demand is coupled to reduced oxygen availability. This is illustrated in Figure 2 by reference to selected cyprinid and salmonid species. Similar effects have been reported of standard oxygen consumption in goldfish (Figures 1 and 3, Table 1) by Beamish and Mookherjii (1964). Their data also indicated that standard oxygen consumption increased in linear fashion with temperature, rising eight-fold between 10° to 35°C. An equivalent increase (~690%) has been reported by Beamish (1964) for carp (Cyprinus carpio) over the same temperature range.

(2) Systemic Responses to Increased Oxygen Demand

In circumstances of decreased oxygen availability and/or increased oxygen demand, fishes may respond by using either or both of two general compensatory modalities. Response at the systemic level is involved with alterations in the branchial-exchanger complex, while that at the cellular level with blood gas transport. This section has been included to examine the former with special emphasis on the inherent limitations involved.

Respiratory system function has been commonly analyzed utilizing the analogy between the gill as a gas transfer system and the operation of a compact, countercurrent heat exchanger. The various parameters involved have been extensively considered under static and dynamic circumstances, both theoretically and under a variety of experimental situations (Rahn, 1966; Randall, Holeton & Stevens, 1967; Taylor, Houston & Horgan, 1968; Randall, 1970; Heath, 1973). However, within the context of the present discussion, attention will be restricted to the effects of only three of the potentially modifiable variables; namely, ventilatory flow, cardiac output and effective exchange surface. A full discussion may be found in recent reviews by Randall (1970b), Shelton (1970), Johansen (1971) and Jones and Randall (1978).

Ventilatory Flow : Responsive increases in ventilatory flow offer an obvious means of amplifying oxygen uptake. Although water viscosity is reduced as the temperature is increased, the teleostean respiratory medium is still dense, viscous and relatively oxygen poor. Therefore, substantial metabolic costs are incurred in order to move adequate volumes of water to meet their oxygen requirements. Estimates of this parameter range from a reasonable 5 to 15% of routine metabolism (Cameron & Cech, 1970) to values as high as 70%. Amplification of ventilatory flow is inherently self-limiting at the point where the cost of extraction exceeds the gain in oxygen acquired and made available to the tissues (Jones, 1971). The cost of operating the branchial pump may become limiting at higher temperatures, and most notably in larger animals. Other problems associated with increased flow include reduction in lamellar exposure and increased shunting of flow through non-exchange areas. In short, the potential benefit of increased ventilation is severely curtailed due to the increased cost of operation and reduced overall efficiency.

Branchial Perfusion : Oxygen uptake may also be enhanced by increasing cardiac output and thus gill perfusion, since virtually all blood pumped by the heart is delivered to the gills. Increased output is achieved through increases in cardiac rate and stroke volume. Costs have not been well-defined, but appear to be less than those associated with increased ventilation (Jones, 1971). Major limitations in cardiac performance do not appear to occur at higher temperatures (Jones, 1971), but may be of importance under cold conditions. In order to achieve effective blood oxygenation, however, cardiac output and ventilatory flow must be closely coupled. In theory, maximum oxygenation occurs when the capacity-rate ratio ($\dot{Q} \cdot \alpha Bo_2$ $[Pao_2 - Pvo_2]$)/ $\dot{V}_I \cdot \alpha Wo_2$ $[P_{Io_2} - P_{Eo_2}]$) (see Table 2) approaches zero and Pa_{o_2} approximates P_{Io_2}. Consequently, cardiac output can only be successfully increased to the point where ventilation becomes inefficient.

Exchange Surface Area : Gill exchange area is another factor involved in increasing oxygen uptake. This can be related to effectiveness of blood oxygenation by means of the branchial transfer unit, $A \cdot d/\dot{Q} \cdot \alpha Bo_2$ (see Table 2). With other factors constant, increases in effective exchange area of the gill improve oxygen uptake. The means by which teleost fishes accomplish this remains controversial. Area amplification can be achieved by channeling of blood flow through respiratory, as opposed to non-respiratory lamellar pathways (Steen & Kruysse, 1964; Richards & Fromm, 1969), or increasing the number of lamellae

TABLE 2 : List of Symbols Used to Analyze Gas Exchange

A	=	exchange area (mm^2)
αB_{O2}	=	blood oxygen capacity (ml $O_2 \cdot L^{-1} \cdot$ mm Hg P_{O2}^{-1})
αW_{O2}	=	water oxygen capacity (ml $O_2 \cdot L^{-1} \cdot$ mm Hg P_{O2}^{-1})
PaO_2	=	arterial oxygen tension (mm Hg P_{O2})
PvO_2	=	venous oxygen tension (mm Hg P_{O2})
P_{IO2}	=	inspiratory oxygen tension (mm Hg P_{O2})
P_{EO2}	=	expiratory oxygen tension (mm Hg P_{O2})
$\dot{Q}$	=	cardiac output ($ml \cdot min^{-1}$)
$\dot{V}_I$	=	ventilatory flow ($ml \cdot min^{-1}$).
$\dot{V}_{O2}$	=	oxygen consumption ($ml \cdot Kg^{-1} \cdot min^{-1}$)
d	=	coefficient of oxygen diffusion across the lamellar barrier ($ml \cdot min^{-1} \cdot cm^{2\ -1} \cdot cm^{-1} \cdot$ 760 mm Hg^{-1})

perfused (Davis, 1972; Cameron, 1974). The latter mechanism is favoured by the results of recent studies.

Systemic responses, however, sometimes induce disadvantageous side effects. It has been thought, for example, that increased muscular activity may so reduce blood pH as to hinder oxygen loading (Powers, 1972).

In addition, water and electrolyte transfer relationships are similar to those of oxygen and because of this, increases in exchange area, ventilatory flow and cardiac output increase rates of endosmosis, branchial efflux and renal loss of electrolytes (Randall, Baumgarten and Malyusz, 1972). Compensation for this secondary stress could be achieved by reductions in lamellar permeabilities and enhancement of branchial absorption and/or renal recovery of electrolytes. Temperature effects upon oxygen consumption, ventilatory flow and diffusional water influx are all comparable (Evans, 1969; Isaia, 1972; Motais & Isaia, 1972), suggesting that water permeability is not greatly altered. Overall branchial electrolyte loss rates rise with temperature, but are substantially less than those seen for endosmosis, suggesting that adaptive reductions in lamellar ion permeability may take place (Maetz, 1972; Cameron, 1976). To compensate for ionic losses, some means of augmenting electrolyte recruitment must be in effect, and consistent with this, increases found in gill, kidney and blood (Na^+/K^+)- and (HCO_3^-)-stimulated ATPase and carbonic anhydrase activities occur (McCarty & Houston, 1977; Houston & McCarty, 1978; Houston & Mearow, 1979a, b; Smeda & Houston, 1979).

In summary, systemic responses involve increased metabolic costs, are inherently self-limiting and/or induce secondary stresses that require compensation. These factors do not negate resorting to this type of response in meeting temperature-induced increases in oxygen requirements, and recent studies (Henry & Houston, 1978-1979, unpublished observations) with rainbow trout, Salmo gairdneri, illustrate their magnitude. Fish acclimated to 18°C exhibited an approximately five-fold increase in resting oxygen consumption as compared to specimens at 2°C. Increases of 125%, 280% and 85% were seen in ventilation rate, ventilatory flow and ventilatory stroke volume respectively. Cardiac rate rose by a factor of almost 3 and electrocardiographic data pointed to increased cardiac output.

Given these considerations, the question arises as to whether adaptive alterations at the hematological level can be utilized to reduce reliance upon more costly systemic alterations and alleviate secondary osmo- and ionoregulatory perturbances. Cameron and Davis (1970) have shown that this may indeed be the case. These investigators examined cardio-vascular-respiratory function after rendering rainbow trout anemic with phenylhydrazine. Reduction in blood oxygen carrying capacity led to major changes in cardiac function, although few effects upon ventilation were seen. Conversely then, enhancement of oxygen transport at higher temperatures could well reduce at least cardiac work.

(3) Hematological Responses to Increased Oxygen Demand

Several forms of adaptive response at the hematological level can be visualized. Oxygen consumption can be considered a product function of cardiac output, blood-oxygen carrying capacity and the arteriovenous oxygen differential (Hughes, 1964), i.e., $\dot{V}_{O2} = \dot{Q} \propto B_{O2} (Pa_{O2} - Pv_{O2})$. Several means by which $\propto B_{O2}$ can be augmented are: (i) amplification of overall hemoglobin content; (ii) reduction in mean erythrocytic volume; (iii) increased erythrocytic numbers ; and (4) elevation of mean erythrocytic hemoglobin content. The effect of the first is obvious. Volume changes are advantageous because the rate of hemoglobin oxygenation varies inversely with red cell volume (Holland, 1970). Increases in erythrocytic numbers would, however, increase viscosity and enhance cardiac work requirement. Red cell hemoglobin concentrations are frequently close to their solubility maxima in the red cells of normal animals (Riggs, 1976) and consequently little scope exists for their further elevation.

A summary of the results of several studies on this aspect of the acclimatory process in goldfish, *Carassius* *auratus*, can be found in Table 3. A noteworthy feature of these data is a lack of consistency despite the fact that experimental conditions were apparently similar. The main types of responses seen include: (i) obviously adaptive responses, i.e. increases in hemoglobin content, hematocrit, red cell numbers and mean erythrocytic hemoglobin and/or reductions in mean erythrocytic volume (Houston & Cyr, 1974; Catlett & Millich, 1976); (ii) absence of response (Anthony, 1961; Houston, *et al*., 1976); and (iii) apparently anti-adaptive responses such as reduction in hemoglobin content, hematocrit and/or erythrocyte abundance (Houston and Rupert, 1976).

A partial explanation for this variability may involve pre-sampling stresses of handling, anesthetization and related procedures (Houston, *et al*., 1969, 1971; Hattingh & van Pletzen, 1974; Fletcher, 1975; Oikari & Soivio, 1975; Soivio & Oikari, 1976), nutritional status (Kamra, 1966; Smith, 1968; Johansson-Sjobeck, *et al*., 1975) and seasonal variations (Denton & Yousef, 1975; Bridges, Cech & Pedro, 1976; van Vuren & Hattingh, 1978). A major factor in present uncertainties stems from our lack of understanding of the factors which regulate erythropoesis in fishes and the extent to which these are influenced by environmental variations.

Another basis for these discrepancies may involve the phenomenon of weight-specific differential hematological

TABLE 3 : Thermoacclimatory modifications in some primary hematological parameters of the goldfish, *Carassius auratus*. Hb, hemoglobin (g · 100 ml^{-1}); PCV, packed cell volume (%); RBC, red blood cells (millions·mm^{-3}); MEV, mean erythrocytic volume (μ^3).

SPECIES	UPPER INCIPIENT LETHAL TEMP.	ACCLIMATION TEMP.	Hb	PCV	RBC	MEV	REFERENCE
Carrassius auratus	38.6°C						Fry, *et al.*, 1942
	36.6°C						Fry, *et al.*, 1946
		5°C 6°C 26°C 30°C		34.2 36.0 37.5 36.9	2.0 2.1 1.7 1.8	172 181 219 176	Anthony, 1961
		2°C 20°C 35°C	4.6 ± 0.17 7.6 ± 0.21 8.4 ± 0.04	30.7 ± 1.04 35.3 ± 0.49 44.7 ± 0.43			Houston & Cyr, 1974
		5°C 30°C	7.9 ± 0.34 6.7 ± 0.26	23.6 ± 1.37 28.3 ± 0.97			Houston, et al., 1976
		3°C 23°C	7.7 ± 1.3 5.6 ± 0.8	31.1 ± 5.5 21.1 ± 3.6			Houston & Rupert, 1976
		1.0°C 5.0°C 10.0°C 21.5°C		28.0 ± 1.96 33.13± 1.28 36.56± 1.96 35.18± 0.51	1.49 ± 0.09 1.78 ± 0.05 1.80 ± 0.06 1.88 ± 0.05	187.92± 8.16 187.36± 7.68 203.53± 7.08 185.42± 4.02	Catlett & Millich, 1976

responses. This is well-exemplified by the findings of Houston and Cyr (1974), Houston and Rupert (1976) and Houston, *et al*., (1976). In small goldfish (13.4 $\pm$ 1.9 to 15.6 $\pm$ 0.6 g) substantial increases in hemoglobin levels were seen following acclimation to a temperature range of 2°C to 35°C. Larger specimens (22.2 to 38.0 g) exhibited only moderate changes while, in still larger animals (36.9 $\pm$ 8.8 to 64.3 $\pm$ 11.4 g), no significant variations were discernible. In fact, mean hemoglobin concentrations were sometimes reduced at higher acclimation temperatures in heavier weight ranges.

Nevertheless, other studies, several on rainbow trout, *Salmo gairdneri*, (DeWilde & Houston, 1967; Houston & Cyr, Houston & Smeda, 1979) and observations on carp, *Cyprinus carpio*, (Houston & DeWilde, 1968; Houston, *et al*., 1976; Houston & Smeda, 1979), suggest a general pattern of hematological response which, when it occurs, involves modest increases in overall hemoglobin content, somewhat larger numbers of red cells of reduced volume and modest elevations in cellular hemoglobin content. These are clearly adaptively-appropriate, but their erratic occurrence and variable magnitude are such as to preclude any major, or centrally important contribution toward the resolution of temperature-oxygen demand problems.

(4) Hemoglobin Polymorphism and the Modulation of Hemoglobin Oxygen Affinity by Temperature, pH, Organophosphates and Inorganic Ions

Hochachka and Somero (1973) have described three major classes of response at the molecular level by which organisms can biochemically adapt to changes in environmental conditions. These involve: (i) variations in the types of macromolecules present; (ii) adjustment in the amounts or concentrations of pre-existing macromolecules; and (iii) adaptive regulation of their functions. They have designated these as 'qualitative', 'quantitative' and 'modulatory' strategies. Available evidence indicates that all occur in the hemoglobin systems of thermally-acclimated fishes. Increases in hemoglobin content consistent with the 'quantitative' strategy were considered in the preceding section.

(A) Hemoglobin Polymorphism

The existence of electrophoretically-distinct hemoglobins in most teleosts has been well-documented (see Table 4). Manwell (1957), with his work on *Scorpanericthys mormoratus* was the first to demonstrate the presence of multiple-component hemoglobin systems in fishes. Ontogenic changes in hemoglobin types and relative abundances have since been confirmed in numerous species (e.g. Vanstone, Roberts & Tsuyuki, 1964; Iuchi & Yamagami, 1969;

TABLE 4 : Electrophoretic Patterns Obtained from Various Species of Fish

SPECIES	NUMBER OF COMPONENTS	ELECTROPHORETIC TECHNIQUE
Carp, *Cyprinus carpio*	3	Starch gel
Carp, *Cyprinus carpio*	3	Sephadex
Goldfish, *Carassius auratus*	3	Paper
Goldfish, *Carassius auratus*	3	Starch gel
Goldfish, *Carassius auratus*	3	Acrylamide gel
Chinook salmon, *Onchorhynchus tshawytscha*	2	Moving boundary
Sockeye salmon, *Onchorhynchus nerka*	7	Starch gel
Chum salmon, *Onchorhynchus keta*	9	Starch gel
Atlantic salmon, *Salmo salar*	9	Starch gel
Rainbow trout, *Salmo gairdneri*	3	Moving boundary
Rainbow trout, *Salmo gairdneri*	11	Starch gel
Rainbow trout, *Salmo gairdneri*	7	Starch gel
Rainbow trout, *Salmo gairdneri*	9	Acrylamide gel
Rainbow trout, *Salmo gairdneri*	6	Cellulose acetate
Brook trout, *Salvelinus fontinalis*	3	Cellulose acetate
Brook trout, *Salvelinus fontinalis*	15	Starch gel
Smallmouth bass, *Micropterus dolomieui*	3	Cellulose acetate
Largemouth bass, *Micropterus salmoides*	4	Cellulose acetate
Catfish, *Parasilurus asotus*	4	Starch gel
Brown bullhead, *Ictalurus nebulosus*	7	Acrylamide gel
Cod, *Gadus morrhua*	3	Agar
Plaice, *Pleuronectes platessa*	5	Agar
Flounder, *Platichthys flesus*	5	Agar
Green sunfish, *Lepomis cyanellus*	6	Starch gel

(from Mearow, 1976)

Perez & Maclean, 1976). Multiple hemoglobin systems are normally characteristic in adults with single-component systems comprising 0% (De Smet, 1978) to 8% (Fyhn, et al., 1979) - 10% (Sharp, 1973) of total species when surveys were conducted. It should be noted that these estimates are conservative in nature for they are based upon electrophoretic mobilities, and thus discriminate only on the basis of net charge in relation to molecular weight and configuration.

The principal question is, however, whether the hemoglobins found in a particular species differ sufficiently in their transport characteristics and/or their sensitivity to hemoglobin-oxygen affinity modulators to offer some adaptive potential. Physiological benefits might well accrue under such circumstances in response to alterations in either oxygen demand or oxygen availability, or both, and would involve selective adjustment in fractional abundancies.

The results of electrophoretic studies must be interpreted with caution, however, for work on human hemoglobins has shown the occurrences of residue substitutions in 'non-critical' regions of the molecule. These are known to alter electrophoretic mobility without appreciably influencing hemoglobin-oxygen affinity and modulator effects (Natelson & Natelson, 1978). Under such circumstances, only relatively minor effects upon oxygen uptake and release are seen.

In the teleosts, examples of both functionally similar and dissimilar hemoglobin families as well as those in which both forms co-exist are known. The killifish, carp and rainbow trout are examples which demonstrate some of the complexities which can occur. In the killifish, Fundulus heteroclitus, the four hemoglobins have been found to be functionally-homogeneous (Mied & Powers, 1978). This was also thought to be the situation in the carp with all exhibiting sensitivities to pH and other modulatory agents (Gillen & Riggs, 1972). However, a recent study (Weber & Lykkeboe, 1978) has shown that altering the proportions of these components without changing the total hemoglobin can significantly influence oxygen affinity. This points to the possibility of a subtle form of affinity adjustment through component interactions not previously anticipated.

The complex hemoglobin system of 7 to 11 component fractions (Houston, et al., 1976) in rainbow trout can be classified into two broad categories (Brunori, 1975). In one, the components are virtually insensitive to pH, are little influenced by inorganic and organic phosphates and exhibit minimal thermosensitivity, while in the other, these factors have a profound influence on oxygen affinity.

The existence of functionally-distinct components within some multiple hemoglobin systems raises the question as to whether the teleosts can affect quantitative or qualitative changes during the thermoacclimatory process. The heat-tolerant goldfish is unique in changing its hemoglobin system in both ways. A two-component system is normally seen under cold conditions ($< \sim 10^{\circ}C$), whereas three fractions are encountered at warmer acclimation temperatures. Apparently, first reported by Falkner and Houston (1966), this phenomenon has subsequently been confirmed in several studies (Houston & Cyr, 1974; Houston, _et al._, 1976; Houston & Rupert, 1976). The two persistent fractions are labelled G_2 and G_3 and the thermolabile variant, G_1. At the higher temperature, G_2, the principal fraction, declined significantly in both actual and proportional concentrations, while G_3 increased and G_1 appeared.

Hemoglobin system changes during thermal acclimation have been examined in several other species. Only limited modifications in the relative and absolute abundances of a restricted range of hemoglobins was exhibited by the white sucker, _Catostomus commersoni_ (Houston, _et al._, 1976) and the brown bullhead, _Ictalurus nebulosus_ (Grigg, 1969). The carp, _Cyprinus carpio_ (Houston, _et al._, 1976) and rainbow trout, _Salmo gairdneri_ (Houston & Cyr, 1974) were both characterized by substantial variation in their components, but none were lost or gained. The pumpkinseed, _Lepomis gibbosus_, if challenged by thermal extremes, may add or delete selected hemoglobins (Houston, _et al._, 1976).

(B) Modulation of Hemoglobin-Oxygen Affinity

Although acclimatory alterations in multiple hemoglobin systems may offer distinct adaptive advantages, these can only be fully appreciated when the accompanying changes in the erythrocytic microenvironment in which they operate are considered. Temperature, pH, organophosphates and a number of inorganic ions constitute four critical factors modulating hemoglobin-oxygen affinity and thus oxygen loading and unloading.

Temperature

Due to the fact that vertebrate oxygen-hemoglobin interactions are exothermic in nature, increased temperature would be expected to reduce affinity and therefore facilitate oxygen release at the tissue level. However, as stated previously, a reduction in oxygen solubility also occurs when water temperatures are raised. Grigg (1969), Eaton (1974) and Powers (1974) have concluded that such an affect would be comparable to hypoxia. Under these circumstances, it was thought that increased oxygen affinity with temperature would be adaptively advantageous.

Thermal effects on the oxygen affinities of teleostean hemoglobins are quite variable (Johansen & Lefant, 1972; Johansen & Weber, 1976). Three general situations have been defined by Weber, *et al*. (1976b): (i) all hemoglobins sensitive to both temperature and pH; (ii) some hemoglobins as in (i) with others having little or no sensitivity to temperature; and (iii) pH-sensitive, temperature-insensitive hemoglobins. The distribution of these hemoglobins has been examined by Johansen and Lefant (1972) who hypothesized that thermal sensitivity is reduced in species normally experiencing moderate-to-large fluctuations in environmental temperature. Powers, et al. (1979) have refuted this generalization using a number of cases, most notably the rainbow trout and killifish, Fundulus heteroclitus.

Weber, et al. (1976b) have examined whole blood hemoglobin oxygen affinities of rainbow trout at their respective acclimation temperatures. In this case, the oxygen affinity of 22°C animals was dramatically reduced in comparison with 5°C specimens, a confirmation of the thermal sensitivities obtained by Eddy (1971) for trout hemoglobins. Additional work by Eddy (1973) has shown that temperature-related oxygen affinity decreases characterize tench, Tinca tinca, hemoglobin. Smeda (1978) has taken the findings of Grigg (1969) and applied the intra-erythrocytic pH conditions reported by Steen and Turitzin (1968) to show that a substantial reduction in affinity in brown bullhead, Ictalurus nebulosus, hemoglobin does occur under in vivo conditions of increased temperature.

The experimental results presented here suggest that teleostean hemoglobins respond to increased temperature such that there is a reduction in hemoglobin-oxygen affinity under in vivo conditions. The net result of this form of adaptation would be a favouring of oxygen release at the tissue level, under circumstances of elevated cellular oxygen requirements.

pH

Reeves (1977) has recently reviewed and extended studies on temperature effects upon acid-base balance in ectotherms. It is well-known that body fluid pH varies inversely with temperature. Two species, carp and rainbow trout, illustrate the effects of such changes on hemoglobin-oxygen affinity. In the former, all hemoglobins are acutely responsive to pH reductions, displaying an approximately 5 fold increase in log P_{50} as pH increases from 7.0 to 7.5 (Riggs, 1970), The Hb IV fraction of the rainbow trout is also characterized by a substantial Bohr effect (Branori, 1975). These changes stem from the effect of hydrogen ions on the internal salt bridges in hemoglobin; increased hydrogen ion strengthening the bridges. This favours the tense configuration of the deoxyhemoglobin molecule over the relaxed oxyhemoglobin state. Such effects operate in conjunction with temperature to decrease affinity at higher temperatures and facilitate oxygen release under circumstances in which oxygen demand is elevated.

Organophosphate Modulation

The effects of organophosphates on hemoglobin-oxygen interactions was first demonstrated in mammals in relation to 2, 3 - DPG (Benesch & Benesch, 1967; Chanutin & Cornish, 1967). This modulator binds preferentially between the NH_2- terminal ends of the β-chains of unliganded hemoglobin molecules (Benesch, et al., 1968; Arone, 1972), introducing additional salt and bridges and stabilizing the deoxy T state of the hemoglobin molecule, with consequent decrease in affinity as measured by readiness of oxygen release.

Since the demonstration of this phenomenon, considerable attention has focussed on the functional role of organophosphates in fishes. The important organophosphates are ATP and GTP, rather than 2, 3- DPG, with GTP more prevalent and more effective than ATP in many species (Geohegan & Poluhowich, 1974; Lykkeboe, et al., 1975; Peterson & Poluhowich, 1976; Torracca, et al., 1977; Bartlett, 1978a, b, c). Binding is thought to occur at the NH_2- termini (Hol, et al., 1978). Some idea of the magnitude of the organophosphate effect can be seen from the work of Tan and Noble (1973) and Gillen and Riggs (1977). In the former, 0.7 mM inositol hexaphosphate added to carp hemoglobin was equivalent to a pH decrease of 1.6 units. Addition of 1 mM ATP to a variety of teleostean hemoglobins was shown, in the latter case, to be equivalent to a 0.5 unit reduction in pH.

Studies on adaptive alterations in red cell organophosphate levels have tended to concentrate on responses of hypoxic stress. These indicate that significant concentration decreases occur (Wood & Johansen, 1972; Wood, et al., 1975; Weber, et al., 1976; Greaney & Powers, 1978) and therefore, an increase in oxygen affinity takes place. Because of this oxygen loading is facilitated. The opposite response is observed in mammals. 2, 3 - DPG levels increase and oxygen delivery to the tissues is facilitated. Organophosphate changes during thermal acclimation have received less attention, but two studies have been carried out. Powers (1974) reported a decrease in the ATP : Hb ratio of catostomid fishes with acclimation to higher temperature. By contrast, Weber, Wood and Lomholt (1976) recorded no significant changes in either ATP or GTP levels in trout red cells following acclimation to 5°C and 22°C. Consequently, the situation in relation to thermal effects is not yet clear.

Ionic Composition and Hemoglobin-Oxygen Affinity Modulation

Studies on mammalian hemoglobin have staablished that a number of inorganic ions have modulating influences on hemoglobin-oxygen affinity, and these have recently been extended to fish hemoglobins.

Chloride

In the case of human hemoglobin, it has been demonstrated that increased chloride concentrations prompt increases in

P_{50}; i.e. reduce affinity (Benesch, et al., 1969; Rossi-Fanelli, et al., 1961b; Antonini, et al., 1962; Bruin, et al., 1974; Rollema, et al., 1975). This effect stems from enhancement of the Bohr effect (an increased number of protons given off upon oxygenation and a lowering of the pH) and also formation of additional salt bridges, favouring the tense, deoxygenated state of the molecule.

Monovalent Cations

Potassium and sodium salts have relatively less effect upon oxygen affinity, but Rossi-Fanelli, et al. (1961b) have shown that potassium reduces oxygen affinity more than does sodium, and this was confirmed by Bunn, et al. (1971). Maximum effect was seen when ATP and not DPG was the principal organophosphate modulator present. This suggests that the differing effects of sodium and potassium on hemoglobin-oxygen affinity are a consequence of differential interactions with ATP and/or chloride.

Divalent Cations

The influence of divalent cations such as magnesium and calcium upon hemoglobin-oxygen affinity is more pronounced than that of monovalent cations. Bunn, et al. (1971) have found that the influence of Mg^{2+} is linked to ATP or DPG. Mg competes with hemoglobin for organophosphates and the interaction is highly conditioned by the oxygenation state of hemoglobin. In the oxyhemoglobin condition, most of the ATP is magnesium-bound, while in the deoxy state, affinity for ATP is increased and the concentration of hemoglobin - ATP is elevated. Bunn, *et al*. (1971) also linked Mg influence to pH. Coupled with the amplified affinity effects due to venous-arterial pH differences, venous pH decreases would tend to protonate ATP, reduce its affinity for Mg^{2+} and, therefore, provide extra phosphate-hemoglobin interaction. The latter would result in the appropriate situation of decreased hemoglobin-oxygen affinity.

Although its action resembles that of magnesium, calcium exerts much weaker effects (Bunn, *et al*., 1971). In addition, calcium concentrations within erythrocytes are low and much of the calcium present is lipid-bound. Consequently, the influence of calcium is slight.

Ionic composition in teleostean erythrocytes has not been examined in detail. A few of the pertinent studies have been summarized in Table 5. Most of these investigations concentrated on monovalent cations and chloride, and only three report thermoacclimatory variations (Catlett & Millich, 1976; Grigg, 1969; Houston & Smeda, 1979). For comparative purposes, human values have also been included in Table 5.

The most extensive study, that by Houston and Smeda (1979) involved the carp, *Cyprinus carpio*, and rainbow trout *Salmo gairdneri*. The results of this study have led to two

TABLE 5 : Representative values (in m-equiv.l^{-1}, packed cells) for red cell electrolyte levels in human and freshwater or freshwater adapted teleosts.

SPECIES	TEMP. (°C)	SODIUM	POTASSIUM	MAGNESIUM	CALCIUM	CHLORIDE	REFERENCE
Human		8.79 ± 1.3	92.7 ± 2.7	4.7 ± 0.7	0.12 ± 0.03	55 ± 5	Natelson & Natelson,1978
Anguilla rostrata	10±1	25.4	46.2			20.3	Munroe & Poluhowich, 1974
Ictalurus nebulosus	9-10	27.0	52.1		0.1		Grigg, 1969
	24-25	48.4	77.1		0.1		
Carassius auratus	1	11.1±0.27	91.3±0.85			94.5±1.19	Catlett & Millich,1976
	5	11.8±0.30	94.7±0.75			102.2±0.69	
	10	11.9±0.24	100.7±1.16			98.9±0.59	
	21.5	14.0±0.27	110.4±2.06			107.3±1.08	
Cyprinus carpio	2 (Spring)		84.5±1.68	16.2 ±0.36	0.74 ± 0.08	45.1 ± 0.85	Houston & Smeda, 1979
	16 (Spring)	1.67±0.56	82.1±2.27	16.8 ± 0.31	0.70 ± 0.08	58.4 ± 1.38	
	30 (Spring)	8.77±0.84	87.3±1.52	11.9±0.47	0.72 ± 0.04	72.5 ± 0.99	
Salmo gairdneri	2 (Summer)	48.9±3.14	98.6±2.66	10.7±0.49	1.11±0.09	100.0±2.20	Houston & Smeda, 1979
	(Winter)	50.5±2.14	105.0±2.48	11.6±1.71	0.60±0.05	94.5±1.89	
	10 (Summer)	50.1±4.57	104.9±2.30	10.8±3.15	1.12±0.11	101.0±1.61	
	(Winter)	44.8±1.68	118.0±1.46	12.1±0.24	0.55±0.05	97.2±2.12	
	18 (Summer)	43.6±2.29	118.5±1.88	10.5±0.48	1.00±0.05	105.0±2.39	
	(Winter)	44.2±1.80	123.0±2.28	12.2±0.28	0.60±0.05	99.0±2.19	

noteworthy findings. These investigators have hypothesized that the pattern of expression is different in the relatively stenothermal trout and the more eurythermal carp, and that under normoxic and hypoxic conditions the response may differ.

In the trout, adaptation appears to involve reduction in hemoglobin-oxygen affinity over its entire thermal tolerance zone. Affinity relationships do not appear to be adjusted through alterations in organophosphate concentration or chloride. Magnesium, Mg^{2+} : Hb ratio and nucloside triphosphate : Hb relationships are not significantly influenced by temperature (Weber, Wood & Lomholt, 1976; Houston & Smeda, 1979). Consequently, it is probable that Mg^{2+} : NTP relationships are also thermostable. Affinity reduction may be accomplished through increases in red cell potassium content linked to reductions in sodium. The key feature in this case, however, is the overall state of the intra-erythrocytic ionic environment. By comparison with the human red cell (see Table 5), and other tissues of rainbow trout (Murphy & Houston, 1977), erythrocytic chloride and potassium levels were relatively high, while those of magnesium were not unusually so (Weber & Lykkeboe, 1978; Houston & Smeda, 1979). Thus, circumstances would tend to reduce affinity. Such a situation provides little scope for response to temperature-induced increases in oxygen requirements, and trout appear to rely principally upon cardiovascular-respiratory responses in resolving the temperature-oxygen demand problem (Heath & Hughes, 1974; Henry & Houston, 1979, unpublished observations). Carp, on the other hand, reduce hemoglobin-oxygen affinity and thereby, facilitate oxygen to the tissues by increasing chloride and Cl^- : Hb ratio while reducing magnesium and Mg^{2+}: Hb ratio.

The response of carp to hypoxia is quite distinct (Weber & Lykkeboe, 1978). Under these conditions, little change in Mg^{2+} : Hb and Pi : Hb occurs, although the GTP : Hb and ATP : Hb ratios are reduced. The sum of GTP, ATP and Pi : Mg^{2+} also declined. Therefore, hemoglobin-oxygen affinity increases through a reduction in nucleoside triphosphate content, both in absolute amount and in relation to magnesium. This contrasts with the response to increases in temperature which are characterized by substantial reductions in magnesium and the Mg : Hb ratio, and large increases in chloride and the Cl^- : Hb ratio decrease hemoglobin-oxygen affinity.

Studies by Catlett and Millich (1976) on goldfish acclimated to 1°C to 21.5°C, found that potassium, sodium and chloride concentrations increased by ~ 20%, ~ 25% and ~ 15% respectively. This suggests a form of response in relation to hemoglobin-oxygen affinity much like that of the carp, (Houston & Smeda, 1979).

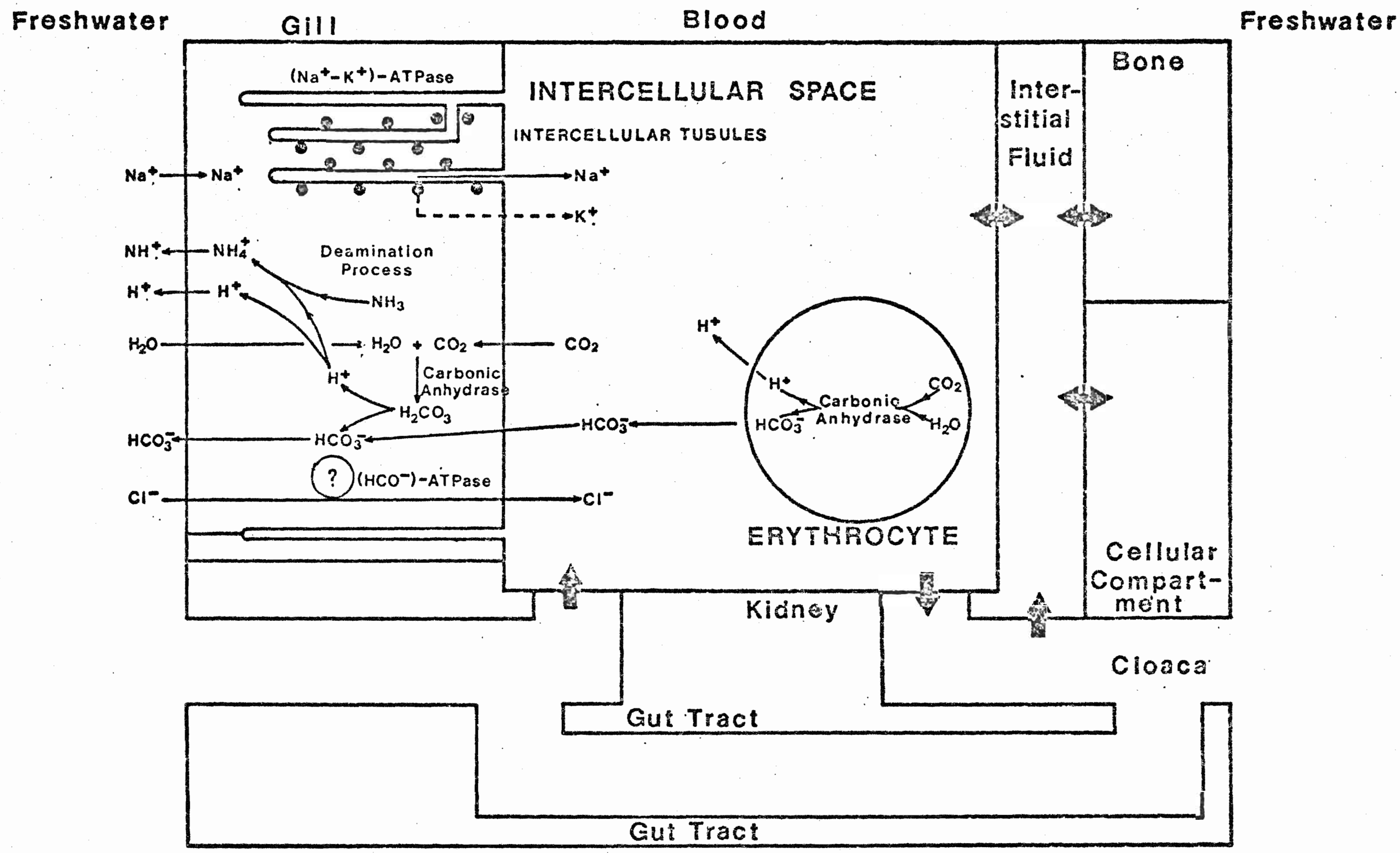
(5) General Features of Ionic Regulation in Fishes

Houston (1973) in his review of water-electrolyte balance in teleosts, has indicated that control involves a variety of mechanisms by which transfer and exchange processes can be governed. These are located at organism-environment interfaces as well as internally, between the various compartments of the body fluid system. These have been outlined in Figure 4. The important areas of exchange with the environment include the gills, kidney and, to a lesser extent, the gut tract. All, in turn, have blood boundaries where exchange can take place. Within the blood itself, transfer takes place between the erythrocytes and plasma. Due to the bulk transport function of the circulatory system, the blood, specifically the plasma, can exchange with bone and the cellular compartment by way of the interstitial and connective tissue fluids. The following discussion will focus on the external exchange processes from the environment through to the blood, as well as to selected internal transfers, and principally those between erythrocytes and plasma. Exchanges involving bone and the cellular compartments will be dealt with very briefly.

The primary function of the kidney in freshwater fishes lies in production of large volumes of dilute urine to reduce overhydration. Accordingly, the nephrons are characterized

Figure 4 : Schematic representation of ionic changes in a typical freshwater teleost emphasizing those located in the gill and blood

(see text for detailed description of processes depicted)



Freshwater
Gill
Blood
Freshwater
(Na^+-K^+)-ATPase
INTERCELLULAR SPACE
INTERCELLULAR TUBULES
Inter-
stitial
Fluid
Bone
Na^+
Na^+
Na^+
K^+
NH_4^+
NH_4^+
Deamination
Process
NH_3
H^+
H^+
H_2O
$H_2O + CO_2$
CO_2
Carbonic
Anhydrase
H^+
H_2CO_3
HCO_3^-
HCO_3^-
HCO_3^-
?
(HCO^-)-ATPase
Cl^-
Cl^-
H^+
H^+
Carbonic
Anhydrase
CO_2
HCO_3^-
H_2O
ERYTHROCYTE
Cellular
Compart-
ment
Kidney
Cloaca
Gut Tract
Gut Tract

by relatively low tubular water permeabilities and active monovalent ion reabsorption systems (Hickman & Trump, 1969). Substantial increases in glomerular filtration rate enable the fish to amplify urine flow, and counteract the increased water influx associated with higher environmental temperatures. This is achieved by increasing the number of functional glomeruli, rather than through increased nephron flow (Mackay & Beatty, 1968). Q_{10} values for urine flow are approximately 2.0 or more in a variety of species, including the carp (Pora & Prekup, 1960; cited in Houston, 1973) and goldfish (Evans, 1969; Mackay, 1974). Because no changes in water reabsorption are seen, tubular water permeability is considered to be independent of temperature (Hickman & Trump, 1969).

Ion absorption by the kidney, although not as well understood as that in the gill, is known to involve (Na^+/K^+) - ATPase and carbonic anhydrase (Hickman & Trump, 1969; McCartney, 1976; McCarty & Houston, 1977). For example, salt recovery is blocked upon carbonic anhydrase inhibition with acetozolamide (Maren, 1967). Active sodium absorption is carried out by (Na^+/K^+) -ATPase. An uncertain proportion of overall chloride reabsorption is believed to be passively associated with this. Potassium can be lost or gained against its concentration gradient (Hickman & Trump, 1967). Houston and McCarty (1978) and McCarty & Houston (1977) have examined the effects of temperature on the activities of these transport systems. Temperature increases resulted in the elevation of enzyme activities when assays were conducted under biologically realistic (i.e., acclimation) temperature conditions.

Urinary electrolyte losses increase substantially at higher temperatures despite ionic reabsorption and consequent urine dilution. In the goldfish (Mackay, 1974), and carp (Houston, 1973), the depletion rates of all ions except potassium rise at high temperature. Thus, the kidney functions effectively in controlling water balance, but cannot maintain electrolyte levels.

Branchial electrolyte losses due to temperature-related increases in ventilation can be reduced in two ways; by limiting this, i.e., reducing permeability, or by increasing rates of recruitment of ions from the environment. Evidence for the former activity has been presented by Maetz (1972) and Cameron (1976). Increases in branchial electrolyte fluxes at higher temperatures were substantially less than those seen in endosmosis, and this suggests the branchial ionic permeabilities are reduced. The latter process is thought to be located in the "chloride cells" of the gill (Maetz, 1971, 1974), involving sites high in ion transport activity and transport enzymes.

Although branchial electrolyte absorption is regarded as an active process, many of the exchanges involved are passive in nature (Maetz, 1971) and thus affect some of the metabolic costs involved with ionic regulation (Houston,1973).

Maetz in 1971 provided an improved chloride cell model of branchial ion uptake through heterionic exchange. This was subsequently modified by Houston and McCarty (1978) to incorporate effects of temperature on the exchanges and transport components of the system. The basic feature of this model involves heteroionic exchanges in uptake of Na^+ and Cl^- and the maintenance of electroneutrality. The exchange ions for Na^+ are NH_4^+, formed from ammonia in the gill, liver and kidney, and H^+, as a result of the reaction : $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$. HCO_3^- fuels Cl^- exchange uptake. Thus, some elements of $Na^+ : NH_4^+$ or H^+ and $Cl^- : HCO_3^-$ uptake are linked through CO_2 metabolism. The exchange sites are believed to be on the mucosal boundary, i.e., the water-cell boundary. On the serosal side, (Na^+/K^+)-ATPases are thought to shift Na^+ out of the cell and K^+ in to make up for the K^+ lost down the concentration gradient. These processes have been demonstrated in goldfish (Maetz & Garcia-Romeu, 1964; DeRenzis & Maetz, 1973), trout (Kerstetter & Mirschner, 1972) and Arctic grayling (Cameron, 1976). An active component, additional to the carbonic anhydrase system, has been proposed as an element in chloride uptake. This may involve a (HCO_3^-)- ATPase (Watson, 1977). A final and non-branchial component of this system is erythrocytic carbonic anhydrase.

At high temperature, the blood carbonic anhydrase and gill and kidney (Na^+/K^+)- ATPase activities are elevated and operate in association with the heteroionic exchange mechanisms to increase uptake. The activities of both systems increase with temperature (McCarty & Houston, 1977; Houston & McCarty, 1978; Houston & Smeda, 1978; Houston & Mearow, 1979). In addition, the rates of both branchial $Na^+/H^+(NH_4^+)$ exchange and Cl^-/HCO_3^- exchange rise with increased acclimation temperature (Cameron, 1976). It is thought that adjustments in HCO_3^- and NH_4^+ concentrations are made in order to increase exchange at high temperatures (Maetz, 1972; Powers, 1974; Cameron, 1976). In summary, Na^+ and Cl^- uptake are enhanced at higher temperatures.

At low temperatures, red cell carbonic anhydrase and branchial and renal (Na^+/K^+)-ATPase become less important due to cold inhibition. However, the gill carbonic anhydrase has relatively low thermal sensitivity (Davis, 1961) and activity remains relatively high and thermostable (McCarty & Houston, 1977). This has the effect of fuelling the Na^+/H^+ exchange, when red cell carbonic anhydrase and ATP-ase activities are negligible. In addition, renal electrolyte recovery may be sufficient for the maintenance of ion levels (Jampol & Epstein, 1970).

Water and electrolyte fluxes within the gut of teleosts has received much less attention. Fleming (1974) indicated that calcium absorption occurs within the intestine, and is mediated by Vitamin D. In addition, the amount of calcium absorbed by fishes is dependent upon the calcium content in the food with increased absorption from the water occurring as the food level is decreased. The urinary bladder has been implicated in sodium and water regulation (Fleming, 1974). The functions of sodium retention and water excretion are carried out in this tissue by active transport of sodium from the mucosal to serosal side in the presence of a permeability barrier which interferes with water movement. In the intestines of goldfish, sodium transport ability is decreased when the temperature is raised (Smith, 1970). This may be a result of the modification of either passive, or active sodium movements or by a combination of both. Smith, et al. (1968) have shown that (Na^+/K^+)-ATPase activity was lower in membranes of warm-adapted fish. Further investigation by Smith and Ellory (1971) gave evidence that the two were not directly related. This was based on the discrepancy between the time taken for adaptation in sodium transport and (N^+/K^+)-ATPase activity. In the latter, the efficiency and not the amount was changed. Iodine has been traced from the food into the thyroid gland of goldfish and back into the bloodstream as a component of thyroxine (Chavin & Bowman, 1965; cited in Phillips, 1969). The absorption and distribution of calcium, phosphorus, cobalt, chloride, sulfate and strontium from the food and to most of the body tissues has been demonstrated for carp (Frolova, 1964; Tominatik & Batyr, 1967; Farberov, 1965; all cited in Phillips, 1969), trout (Phillips, et al., 1958, 1969, 1960a, 1961; Podoliak & McCormick, 1967; all cited in Phillips, 1969) and other species (Nelson, 1961; Templeton & Brown, 1963; Smelova, 1962; Ichikawa & Oguri, 1961; Hunn & Reineke, 1964; Schiffman, 1961; all cited in Phillips, 1969).

Exchanges between erythrocyte and plasma have only recently been investigated. Refuting earlier studies by Haswell and Randall (1976, 1978), Cameron (1978) provided evidence that a chloride shift does occur in fish and that the carbonic anhydrase of the red blood cell is available to plasma HCO_3^-. This mechanism of exchange is depicted in Figure 4. Respiratory CO_2 entering the plasma results in an uncatalyzed equilibrium producing bicarbonate. This then diffuses into the red cell where it is rapidly dehydrated by carbonic anhydrase. In response to inward diffusion of HCO_3^-, Cl^- ions diffuse out in order to maintain electroneutrality. However, this exchange is small in comparison to that provided by the following mechanism. CO_2 entering the plasma diffuses rapidly into the erythrocyte (Forster, 1969; cited in Cameron, 1978) where carbonic anhydrase quickly causes its hydration and the subsequent formation of bicarbonate and hydrogen ion. The bicarbonate thus formed diffuses out of the cell and back into the plasma. In response to this transfer, Cl^- moves into the cell. The net effect is an increase in Cl^- concentration within the erythrocyte.

This system has a dramatic effect on internal CO_2 content and its elimination to the environment. The conversion of most of the CO_2 into HCO_3^- reduces the pCO_2 of the blood in relation to the tissues and, therefore, allows more CO_2 to enter the plasma from the tissues. This results in the maintenance of low internal pCO_2's and an enhanced CO_2 carrying capacity in the blood.

At the gills, CO_2 enters the "chloride cell" as HCO_3^- and CO_2. The latter can be excreted directly down its concentration gradient or it can be converted by carbonic anhydrase to additional HCO_3^- and H^+. Therefore, a two-fold purpose is served by this system; 1) carbon dioxide is effectively removed and 2) the Na^+ and Cl^- uptakes are fuelled.

The effect of increased temperature on erythrocytic carbonic anhydrase activity has been previously referred to under the consideration of electrolyte exchange at the gill. In summary, increased activities (McCarty & Houston, 1977; Houston & McCarty, 1978; Houston & Mearow, 1979; Houston & Smeda, 1979) compensate for the elevated CO_2 production as well as aid in electrolyte recruitment at higher temperatures.

Houston and Smeda (1979) found a reciprocal relationship between erythrocyte chloride and magnesium levels. This was most noticeable in the case of carp acclimated to high temperatures. They hypothesized that the most likely mechanism was a passive redistribution of magnesium as a consequence of temperature-related changes in membrane potential, (as indicated by chloride equilibrium potentials (Dalmark, 1976)). This is an attractive scheme considering there is little evidence of active magnesium transport across the red cell membrane (Christensen, 1975). The appropriate inverse relationship between magnesium content and cellular Cl^- and membrane potential necessarily follows.

(HCO_3^-)- and (Na^+/K^+)-ATPase activities in the rainbow trout erythrocyte were examined by Houston and Mearow (unpublished observations). The former would presumably be involved in extrusion of bicarbonate from the red cell with possible reductions in intracellular pH conducive to Bohr effects. The latter, by analogy with the mammalian system (Christensen, 1975), would be associated with export of sodium and influx of potassium. In the trout, (Na^+/K^+)-ATP-ase activity increases with acclimation temperature. However, Ca^{2+} inhibits this enzyme from the inner side of the membrane (Christensen, 1975) and Houston and Mearow (1979; unpublished observations), proposed that depletion of cellular Ca^{2+} at higher temperatures is linked to enhancement of membrane (Ca^{2+})-ATPase activity. The resultant effect would be a deinhibition of the (Na^+/K^+)- stimulated system with consequent changes in cellular potassium and sodium content.

Like that of (Na^+/K^+)- ATPase, the activity of the HCO_3^- stimulated enzyme, was enhanced at increased temperatures. This would augment transfer of CO_2 by the blood and also contribute to ion uptake at the gill.

In summary, the teleostean erythrocyte is equipped with a variety of active transport systems which are, in most cases, thermosensitive. Cl^- influx via the chloride shift is mediated by carbonic anhydrase, while Na^+, K^+, HCO_3^- and Ca^{2+} movements are governed by the activities of the ion-stimulated -ATPase systems. The only passive transfer is apparently that of Mg^{2+}. In this case, Mg^{2+} efflux may result from inward movement of Cl^- and a consequent change in red cell membrane potential.

Fleming (1974) has reviewed the role of bone in teleostean calcium metabolism. Bone serves as a storage depot for Ca^{2+} and can release it as required to enhance calcium availability. The hormones, calcitonin and parathyroid hormone, control storage and resorption processes with the latter stimulating resorption and mobilizing calcium and phosphate into plasma. Although the mechanism of calcitonin action is not well understood, it seems clear that it inhibits parathyroid hormone action.

Transfers between plasma and intracellular fluid involve passage through the interstitial fluid and occurs at the micro-circulatory level. Holmes and Donaldson (1969) have provided a concise description of these exchanges. The net movement of fluid out of the capillaries stems from the forces of filtration and absorption along the inside and outside of the capillaries (based on the Starling hypothesis). Fluid filtration out of the capillaries is greater at the arterial end, while osmotic recovery of fluid from interstitial spaces is pronounced at the venous end. The forces governing the former activity include capillary hydrostatic pressure (CHP) and the interstitial fluid oncotic pressure (IOP). The latter is controlled by the interstitial hydrostatic pressure (IHP) and the plasma oncotic pressure (POP). The net filtration or absorption force at any point along the capillary is indicated by the relationship : (CHP + IOP) - (IHP - POP). Passage of blood through the capillary results in a continuous transcapillary efflux of water and solutes out of the plasma. In general, the plasma oncotic pressure (POP) increases while the capillary hydrostatic pressure (OHP) decreases as blood passes from the arterial to the venous end of the capillary loop. The former is a result of the limited permeability of the capillary wall (no protein efflux) and the latter, a reduction in the plasma volume.

The permeability characterizing the capillary and cellular membranes is such that a Donnon equilibrium is created between the intracellular and interstitial fluid. Therefore, the exchanges seen between the cells and their bathing fluid conform to this situation.

In summary, the various exchange processes, both externally and internally, result in the electrolyte distributions seen throughout the teleostean body. This can be modified by changes in the thermal environment of the fish.

(6) The Nature of Thermoacclimatory Electrolyte Responses in Freshwater Fishes

Most of the work on thermal effects on water-electrolyte levels has centered on the concentration changes of plasma and skeletal muscle. Information on other tissues, although scarce, is nevertheless important and contributes to our understanding of the acclimatory process.

Temperature-induced alterations in the water content of many tissues are characterized by two features: 1) decreases in water moisture levels with increases in temperature; and 2) variations seen are minute in relation to changes in water influx (Houston, *et al*., 1968; Murphy & Houston, 1977; Mearow & Houston, 1979; unpublished observations). The sensitivity of water distribution to temperature, however, is much greater. In most cases, there is an increase in extracellular phase volume, coupled with a decline in the volume of the cellular compartment as temperature increases. This appears to be a reflection of the modifications in cellular organic solute levels rather than the changes in extracellular composition or cellular electrolyte concentrations (Mearow & Houston, 1979; unpublished observations).

Changes in plasma ion concentrations with temperature are depicted in Figures 5 and 6 for representative eury- and stenothermal, freshwater or freshwater adapted fishes. Anticipated decreases with increases in temperature in the concentrations of both the major (sodium and chloride) and the less abundant (potassium, magnesium and calcium) electrolytes are rarely encountered. Three general patterns can be distinguished in the available information: 1) stable values over much of the tolerance zone; 2) a sharp increase with concentration at moderately low temperatures, with levelling off at intermediate to high temperatures; and 3) an intermediate high above those seen under either cold or warm conditions.

The effects of temperature on cellular ion levels is not as well documented as those of plasma. Given the importance of enzyme modulation by inorganic ions (e.g. Bygrave, 1967), this severely limits understanding of many metabolic features of the acclimatory process. Recent investigations on muscle, liver and a range of other tissues in rainbow trout (Murphy & Houston, 1977; Mearow & Houston, 1979, unpublished observations) have, however, expanded our knowledge in this

Figure 5 : Plasma osmolality ($mOsm \cdot l^{-1}$), sodium ($mmol \cdot l^{-1}$) and chloride ($mmol \cdot l^{-1}$) levels in relatively stenothermal and eurythermal teleosts following constant temperature acclimation.

(taken with permission from Houston, 1980)

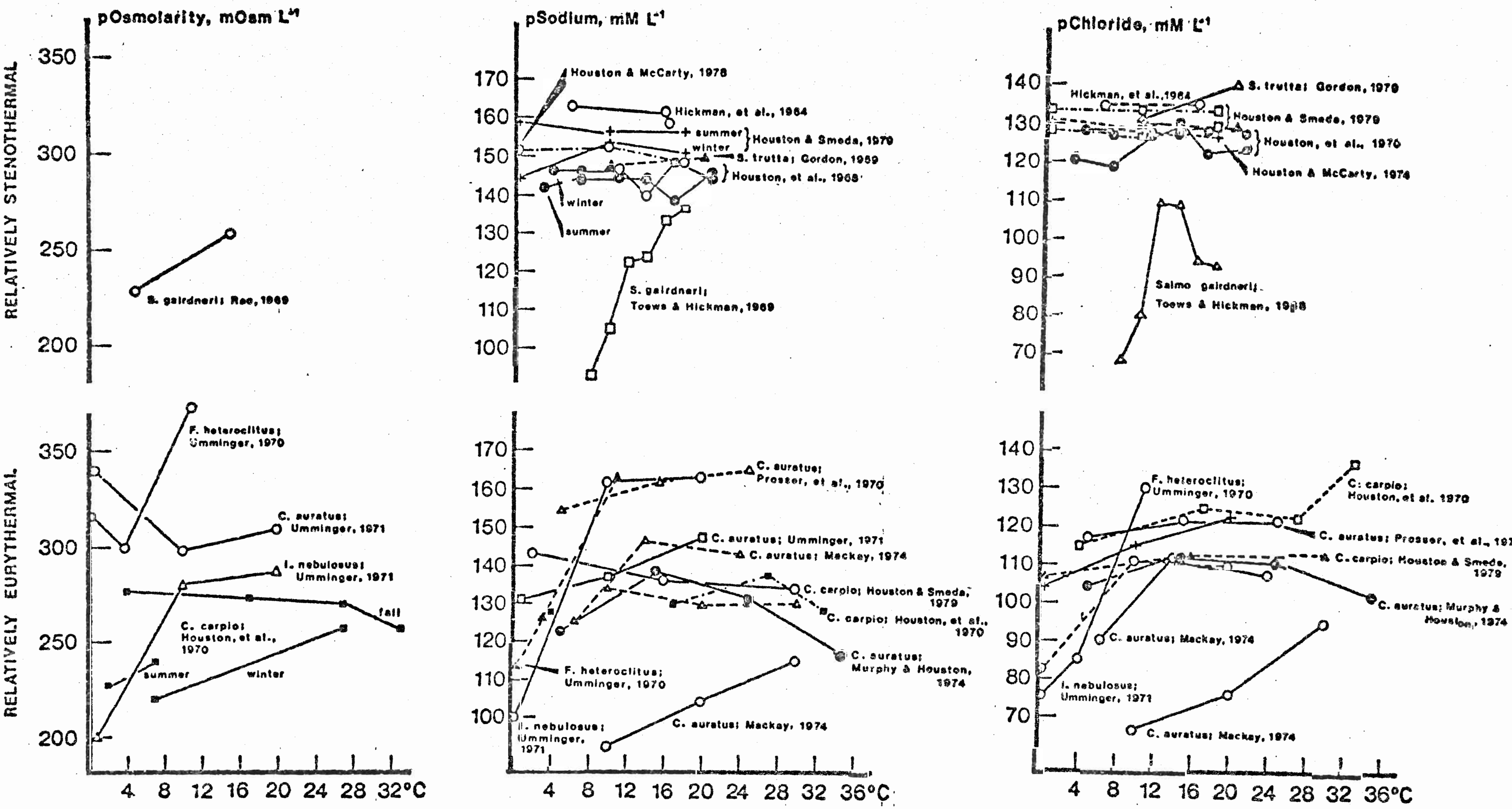
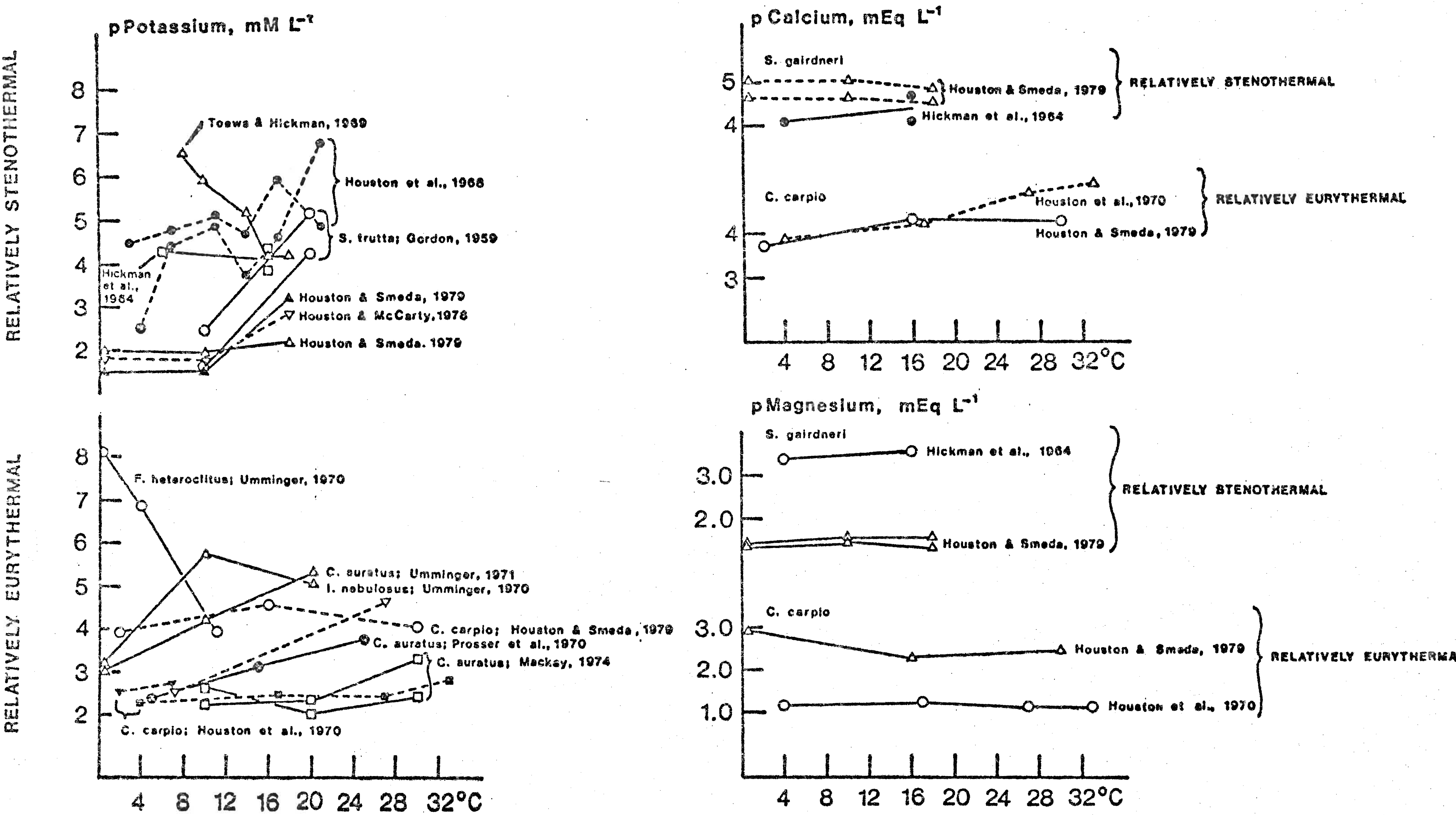

pOsmolarity, mOsm L⁻¹
pSodium, mM L⁻¹
pChloride, mM L⁻¹
RELATIVELY STENOTHERMAL
RELATIVELY EURYTHERMAL
S. gairdneri; Rao, 1969
Houston & McCarty, 1978
Hickman, et al., 1964
summer
winter
Houston & Smeda, 1979
S. trutta; Gordon, 1959
Houston, et al., 1968
S. gairdneri; Toews & Hickman, 1969
S. trutta; Gordon, 1979
Houston, et al., 1970
Houston & McCarty, 1974
Salmo gairdneri; Toews & Hickman, 1968
F. heteroclitus; Umminger, 1970
C. auratus; Umminger, 1971
I. nebulosus; Umminger, 1971
C. carpio; Houston, et al., 1970
fall
C. auratus; Prosser, et al., 1970
C. auratus; Mackay, 1974
C. carpio; Houston & Smeda, 1979
C. auratus; Murphy & Houston, 1974
C. carpio; Houston, et al. 1970
C. auratus; Prosser, et al., 1970
C. auratus; Murphy & Houston, 1974
4 8 12 16 20 24 28 32°C
4 8 12 16 20 24 28 32 36°C

Figure 6 : Plasma potassium ($mmol \cdot l^{-1}$), calcium ($m\ Eq \cdot l^{-1}$) and magnesium ($mEq \cdot l^{-1}$) levels in relatively stenothermal and eurythermal teleosts following constant temperature acclimation.

(taken with permission from Houston, 1980)



RELATIVELY STENOTHERMAL
RELATIVELY EURYTHERMAL
pPotassium, mM L-1
Toews & Hickman, 1969
Houston et al., 1968
S. trutta; Gordon, 1959
Hickman et al., 1964
Houston & Smeda, 1979
Houston & McCarty, 1978
Houston & Smeda. 1979
F. heteroclitus; Umminger, 1970
C. auratus; Umminger, 1971
I. nebulosus; Umminger, 1970
C. carpio; Houston & Smeda, 1979
C. auratus; Prosser et al., 1970
C. auratus; Mackay, 1974
C. carpio; Houston et al., 1970
4 8 12 16 20 24 28 32°C
p Calcium, mEq L-1
S. gairdneri
Houston & Smeda, 1979
Hickman et al., 1964
RELATIVELY STENOTHERMAL
C. carpio
Houston et al., 1970
Houston & Smeda, 1979
RELATIVELY EURYTHERMAL
pMagnesium, mEq L-1
S. gairdneri
Hickman et al., 1964
RELATIVELY STENOTHERMAL
Houston & Smeda, 1979
C. carpio
Houston & Smeda, 1979
RELATIVELY EURYTHERMA
Houston et al., 1970

area. In the case of muscle and liver, interactions between temperature and photoperiod were considered while in the latter, only temperature effects were examined. Season, photoperiod and temperature were all implicated in the compositional variations seen. Qualitatively, modifications within the cellular compartment were similar to those encountered in the plasma, but of reduced magnitude. Excitable tissues (swimming muscle, cardiac muscle, brain) were the most notable in this respect, while metabolically-active tissues (liver, gut, spleen) exhibited more variation.

Lethal temperature shock experiments involving cold or heat exposure, yield similar patterns of response. These include elevated water content, phase shifts of water and reductions in plasma and tissue electrolyte levels (Houston, 1962; Heinicke & Houston, 1965a). Abrupt sublethal transfers have been performed on rainbow trout (Hickman, et al., 1964; Reaves, et al., 1968) and goldfish (Heinicke & Houston, 1965b). The results obtained are similar to those involved in lethal shock studies. Major plasma electrolytes were transiently reduced, tissue ions displayed more variability but frequently increased, and cellular-to-extracellular phase water shifts took place. Such studies have clarified two important features in application of thermal stresses. The first pertains to the rate at which the stress is imposed upon the organism. This is critical in terms of the patterns of changes in the concentration and distribution seen. The second factor is such that the magnitude of applied stress is more important than its quality. The scant amount of information in this area suggests the need for more studies on a greater variety of species.

(7) Cycling Temperature Studies

It has been long recognized that freshwater environments are not usually thermostable. Although seasonal fluctuations have been noted (e.g. Dewilde & Houston, 1967), most investigations have concentrated on examining physiological parameters in relation to static temperature situations. Very few studies have been undertaken to assess the effects of diel temperature cycles on the physiology of aquatic organisms.

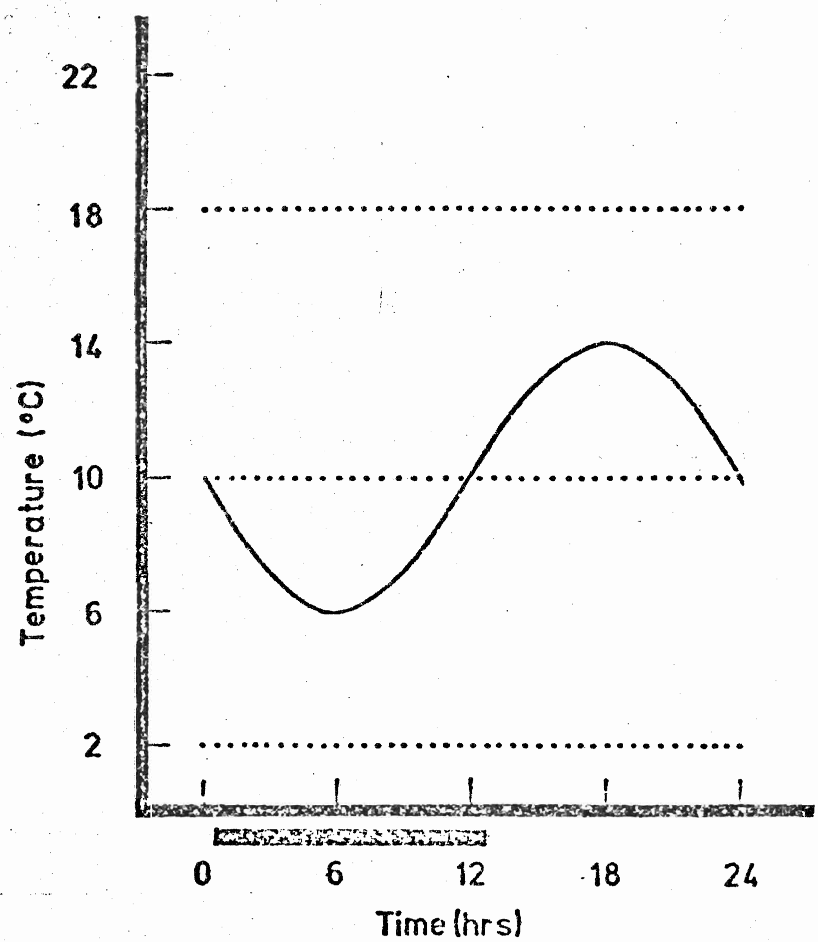
Studies involving invertebrates were performed by Widdows (1976) and Dame and Vernberg (1978). In the former, exposure to cyclic temperatures resulted in an increased temperature independence of the oxygen consumption and filtration feeding rates within the range of the fluctuating regime. Furthermore, this was attained only in animals obtained from environments characterized by marked diel alterations. A cycling thermal environment which extended above the normal environmental maxima resulted in respiratory adaptation and maintenance of feeding rate. This was not seen under constant thermal conditions. Dame and Vernberg (1978) found that, by comparison with constant temperatures, the cyclic condition produced significantly lower oxygen consumption rates in the 15°C to 25°C range. This depression occurred over that portion of the yearly temperature range within which the animals were most active, thus suggesting a more efficient utilization of energy.

The effects of diurnal temperature changes upon fishes appear to have initially emphasized temperature tolerances. Heath (1963), for example, observed that in response to square-wave cycles, sea-run cutthroat trout (*Salmo clarki clarki*) had increased heat tolerance, i.e., they appeared to be acclimating to high temperature components of the cycle. Feldmeth, *et al*. (1974), found that the heat tolerance of the desert pupfish, *Cyprinodon nevadensis amargosae*, was significantly increased by acclimation to cycling temperatures. R. W. Threader, in this laboratory, has recently shown (1979, unpublished observations) that rainbow trout, *Salmo gairdneri*, exposed to cycling temperatures ($12 \pm 4°$, $12 \pm 7°$C) developed resistance equivalent to specimens acclimated to constant temperatures of ~13.5° and 14.5°C respectively. Growth studies by Spieler, *et al*. (1977) indicated that heat applied during the last 4 hours of darkness was particularly conducive to weight gain and testicular growth in the goldfish, *Carassius auratus*. Biette and Green (1980) examined the growth of underyearling sockeye salmon (Oncorhynchus nerka) exposed to a cyclic temperature similar to that encountered during their diel vertical feeding migrations within lakes and reported more rapid and efficient growth than under conditions of constant temperature.

Henry (1980) presented a detailed study into the effect of diurnally cycled temperatures on the cardiovascular-ventilatory functions in statically-acclimated rainbow trout, Salmo gairdneri. His method involved acclimating fish to either a cycled temperature regime or one of three static conditions (Figure 7). Various ventilatory cardiovascular rate functions were determined under both conditions, as well as one in which the static animals were exposed to a diurnal temperature cycle for the first time (Figure 8). In general, the response of trout acclimated to cycling temperatures was similar to that of trout acclimated to constant temperatures and exposed to the cycle for the first time. This is illustrated in the oxygen consumption curves obtained from the study (Figure 9). This parameter was found to increase and decrease with temperatures of the cycle through changes mediated by adjustments in ventilatory flow, rate and stroke volume. Cardiac rate, percent utilization and gill resistance, were also shown to vary in a similar manner, although, at higher temperatures, this was not observed in 18°C animals. In fact, the above data indicate that 18°C statically—

Figure 7 : Thermal acclimation regimes used by Henry (1980). Solid curve represents the cycling temperature regime. Broken lines denote the constant temperature regimes. The horizontal black bar represents the dark period of the 12/12 L/D photoperiod.

(taken with permission from Henry, 1980)



22
18
14
10
6
2
Temperature (°C)
0
6
12
18
24
Time (hrs)

Figure 8 : Experimental tests used by Henry (1980). Solid curve represents the cycling temperature regime. Broken lines denote the constant temperature regimes. The horizontal black bar represents the dark period of the 12/12 L/D photoperiod.

(taken with permission from Henry, 1980)

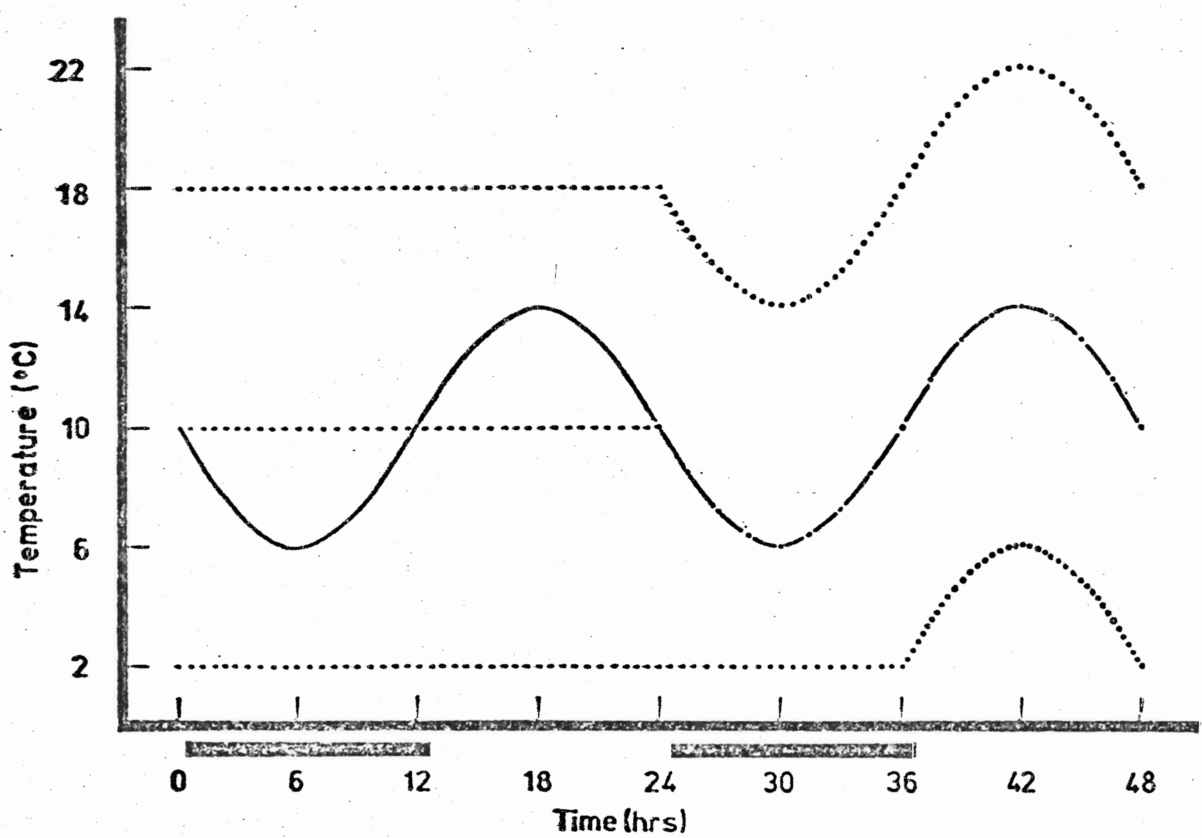

22
18
14
10
6
2
Temperature (°C)
0
6
12
18
24
30
36
42
48
Time (hrs)

Figure 9 : Effect of temperature on oxygen consumption (VO_2, mg Kg^{-1} hr^{-1}) in the rainbow trout, *Salmo gairdneri*.

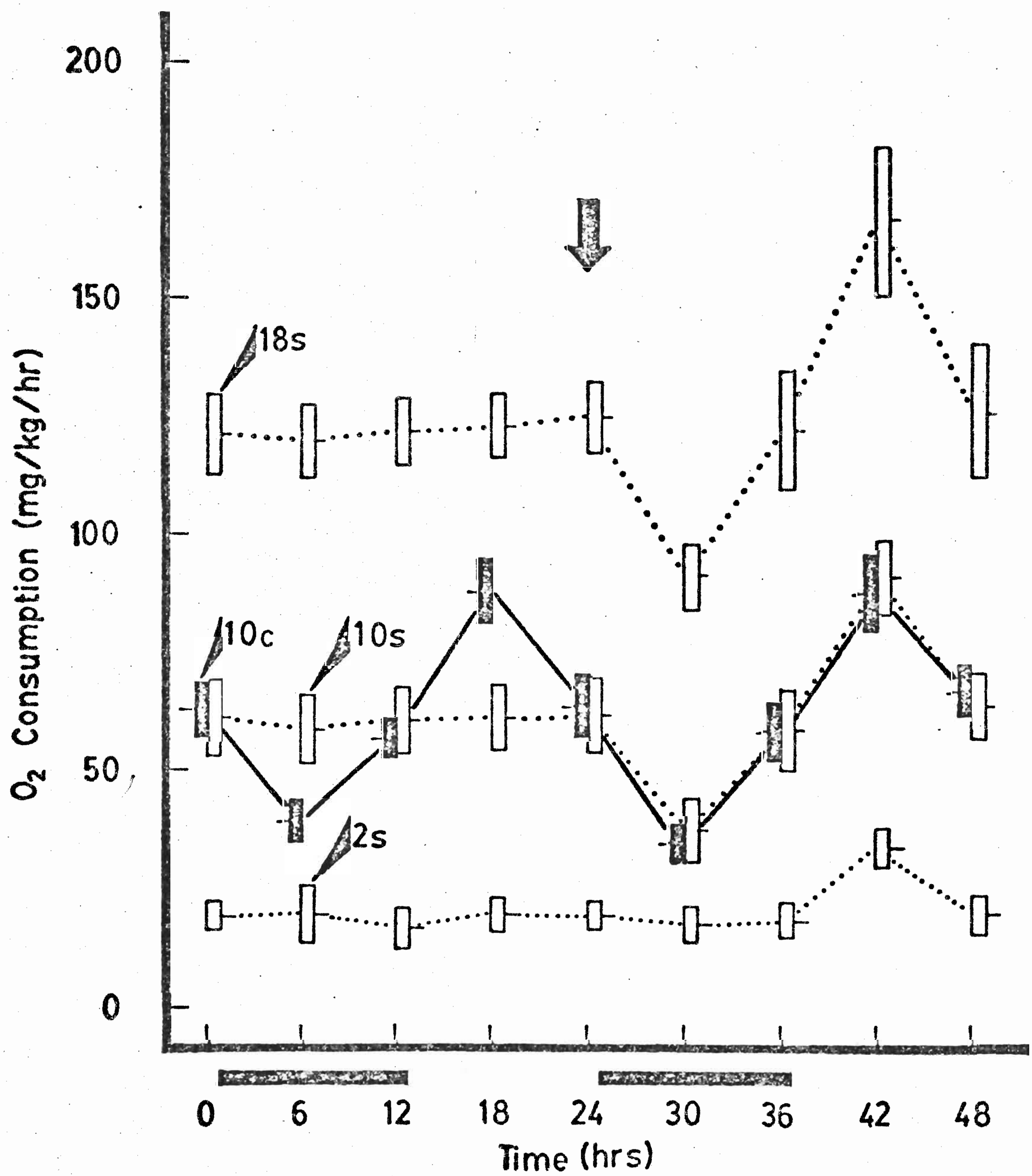
Vertical bars above and below the mean values represent ± 95% confidence intervals of the mean. The open vertical bars represent values for fish acclimated to a constant temperature (25° - 2°C, 10s - 10°C, 18s - 18°C).

The closed vertical bars represent values for fish acclimated to a diurnal temperature cycle (10c).

The closed horizontal bars represent the dark period of the 12/12 L/D photoperiod.

The arrow indicates the onset of the diurnal temperature cycle for the statically-acclimated fish. 2°C constant temperature fish were only subjected to the upper portion of the cycle (36 to 48 hr).

(taken with permission from Henry, 1980)



200
150
100
50
0
O_2 Consumption (mg/kg/hr)
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

acclimated fish were highly stressed when exposed to higher temperatures of the cycle.

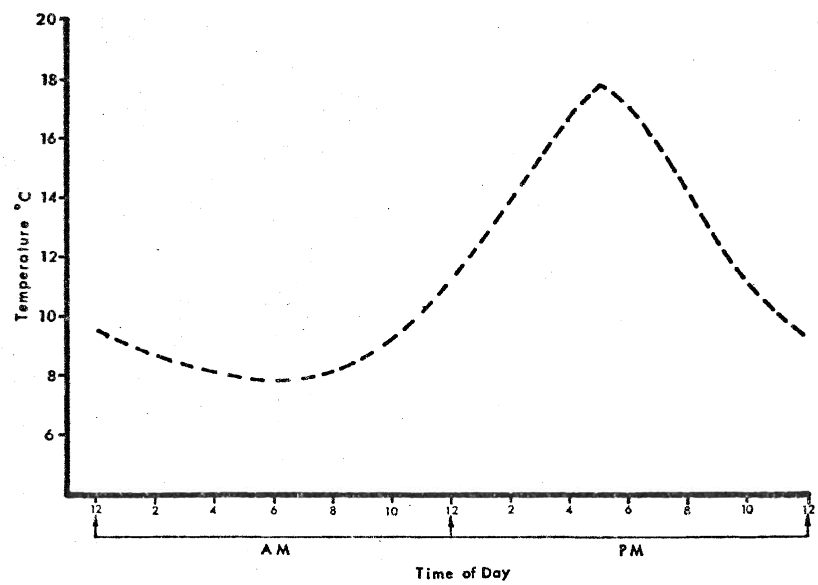
The author hypothesized that both groups of fish may have been acclimated to a similar thermal range, regardless of the acclimation regime employed. Such a scheme would seem to be more tenable than the hypothesis that these animals exhibited no ability to thermally acclimate.

In the context of the present investigation, the work of Toews and Hickman (1969) on electrolyte balance in rainbow trout (*Salmo gairdneri*) is of particular interest. Several features of their experimental technique, however, require some consideration. The application of their cycle was such that fish did not receive equal exposure to high and low temperatures (see Figure 10). The significance of this was that the fish were subjected to pulse-like periods of heat which resembled spikes in the temperature-time curve. Secondly, sampling was carried out at 2.0°C intervals which, due to the nature of the cycle, produced a clustering effect about the high temperatures. Finally, control groups were only sampled at times corresponding to the temperatures of the cycle, with no regard to the possibility of intrinsic fluctuations under any one of the static regimes.

Although the above factors represent major flaws in experimental design, these data still provide some interesting results. In the context of cycled versus static thermal

Figure 10 : Diurnal temperature cycle employed by Toews and Hickman (1969).

(Adapted from Toews and Hickman, 1969)



conditions, the following points are noteworthy: 1) at temperatures above 14°C, muscle water of control fish decreased significantly, a feature not observed in cycled animals; 2) plasma water content of both cycled and control fish remained stable with temperature; 3) plasma sodium, potassium and chloride levels in cycled fish were similar to those of control specimens held at 8° and 10°C; and 4) sodium and potassium in the muscle of cycled fish displayed a marked degree of variability whereas, in the controls, the former was stable and the latter peaked at 14°C. In addition, a highly significant inverse relationship was obtained for cycled temperature sodium and potassium levels of muscle. The foregoing results indicate that the cycled trout acclimated to the lower temperature of the cycle. This is not surprising considering the nature of the cycle employed.

MATERIALS AND METHODS

1. Origin and Maintenance of Experimental Animals

Goldfish, *Carassius auratus*, were obtained from the commercial suppliers : 1) Hartz Mountain, Rexdale, Ontario; 2) Tropic Aquaria, Brampton, Ontario and 3) Carolina Biological, Burlington, N.C. Additional fish were obtained by seining a local drainage (Virgil Dam Reservoir, Virgil, Ontario) during the summer and fall of 1978. The specimens in this investigation had a mean weight of 32.56 $\pm$ 1.11 gm. The sampling of experimental animals commenced on February 13, 1978 and ceased on February 4, 1979.

Upon arrival in the laboratory, the fish were inspected and, if judged to be in good condition, placed in a Model RT-430 Frigid Units Inc. 1000 l fibreglass tank equipped with a Model BHL-1076 recirculating refrigeration unit and a continuous dechlorinated water supply. The latter provided regulation of water temperature, cycling and splash aeration. Oxygen levels were 80% of saturation or better, dependent upon water temperature. Holding tank temperatures were initially adjusted to shipment water temperature or of the water from which the fish were caught to minimize the risk of thermal shock.

After normal feeding and swimming activities had been resumed, the specimens were transferred to experimental 200 l, insulated fibreglass tanks. The dechlorinated water in these units was circulated at $\sim 5\ l \cdot min^{-1}$ through filters containing polyurethane foam filter pads. The tanks were equipped with light-tight photoperiod hoods, and all acclimations carried out on a 12 hr - 12 hr light-dark photoperiod.

Initially, water temperatures in experimental tanks were identical to that of the holding tank. After return to normal activity and feeding, they were adjusted $\sim 1.0°C$ day^{-1} until the desired static temperatures (20°, 25°, 30°C) were attained. In the case of the animals exposed to the cycling temperature regime (25° ± 5°C), midpoint temperature was first established before cycles were initiated.

Water temperatures were regulated using thermistor-equipped duty-operated regulators designed and constructed by J. Rustenburg. These were linked to 1000 watt stainless steel immersion heaters, and provided control to within ± 0.1°C of set-point (verified with a U.S. National Bureau of Standards certified thermometer). Diurnal temperature cycles were established by linkage of a low frequency sine wave generator (J. Rustenburg) with a thermistor-equipped regulator. The regulator, in turn, was coupled to a 1000 watt stainless steel heating coil, and a freezer unit. The latter pumped antifreeze solution through a stainless steel coil submerged in the tank. With this apparatus, a sine wave diurnal temperature cycle, accurate to within 0.5°C at any time, was obtained.

Photoperiod hoods were equipped with 40 watt light receptacles in order to provide 11-18 foot-candles light intensity at the water surface. A photoperiod of 12 hr light : 12 hr dark, controlled within 10 minutes was established by means of Intermatic T-101 time switches. Photoperiods were set so that on and off periods occurred 2 hours prior to sampling, i.e., at 07:00 hr and 19:00 hrs.

Fish were fed daily *ad libitum* on a commercial diet of Purina Trout Chow (Purina Industries). Excess food was removed after the animals had ceased active feeding. Filtering and pumping systems were cleaned, and water volumes maintained at constant levels by addition of dechlorinated water as required.

Minimum acclimation periods of 3 weeks were used prior to sampling; this period being based on the results of thermal shock experiments (Heinicke & Houston, 1965b; Reaves, *et al*., 1968) and the time-course studies of Sidell, *et al*. (1973).

No mortalities occurred during acclimations and the animals appeared to be healthy.

2. Sampling Procedure

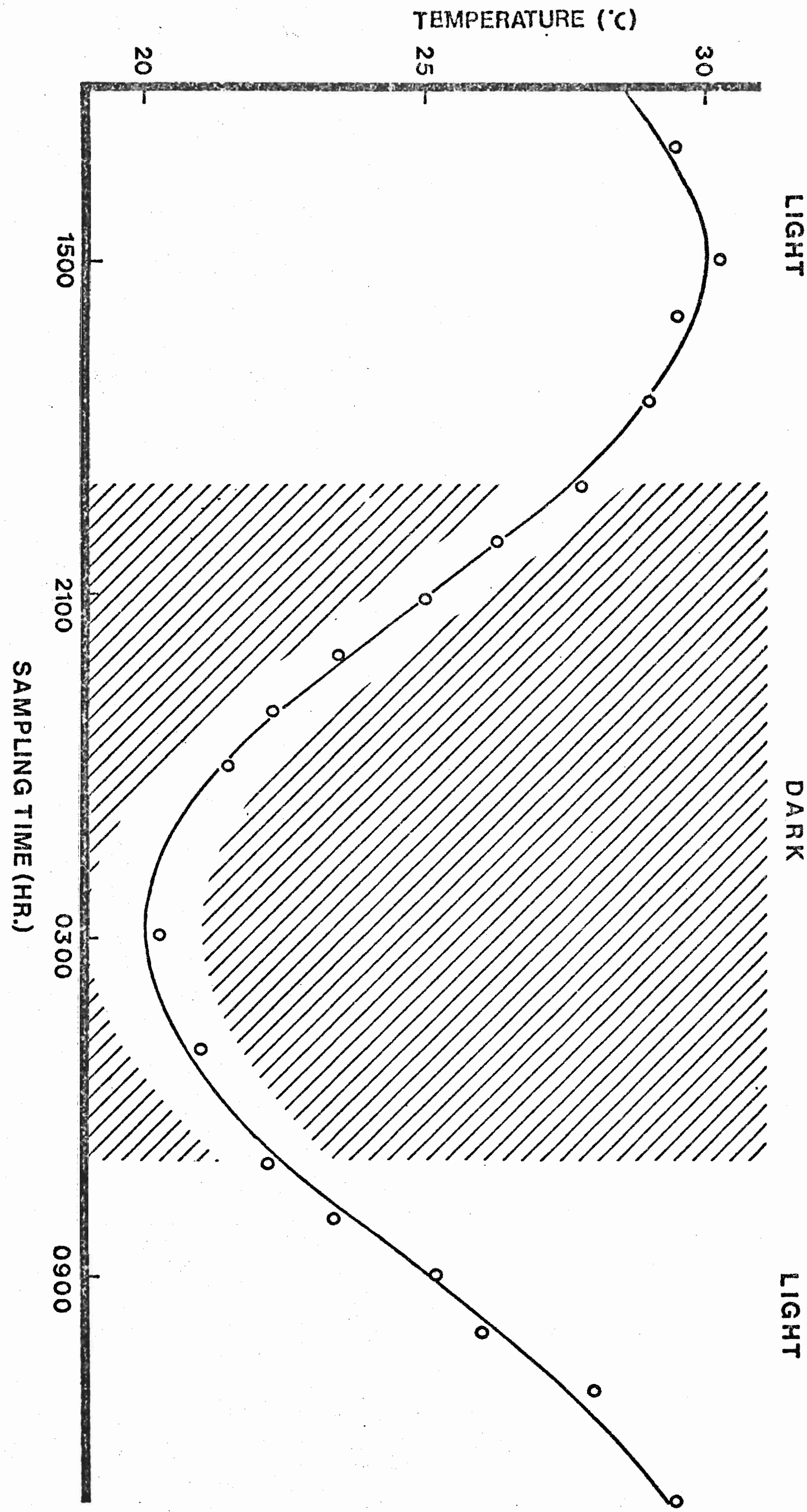
A) Time of Sampling

Sampling was carried out at times corresponding to the maximum, mid-point and minimum temperatures of the diurnal cycle, i.e., at 03:00, 09:00, 15:00, and 21:00 hrs (Figure 11) under both cycling and constant temperature regimes. The latter regime was, of course, essential to differentiate between variations of a diurnal nature and those resulting from temperature *per se*.

B) Blood Sample

At appropriate times, Individual fish were netted, wrapped in damp papertowelling, and the ventral caudal peduncle scaled. Blood was drawn from the caudal vessels in ammonium-heparinized (Sigma Chemical Co., St. Louis, Mo., 50,000 units) 1 ml syringes equipped with 23 gauge needles. Following this, the blood was divided into 2 portions. The major portion was transferred into a capped 1.0 ml disposable centrifuge tube (Fisher Scientific Co., Don Mills, Ontario), and immediately centrifuged (5000 g, 5 min) in a Fisher micro-centrifuge (Fisher Scientific Co., Don Mills, Ontario). The plasma was then removed with a capillary pipette and stored in a plastic, sealed vial at -80°C. A thin layer of plasma was left on the surface of the packed erythrocytes still contained within the centrifuge tube. This procedure

Figure 11 : Cycling thermal regime employed in the study. Curve denotes a theoretical sine wave temperature function. Open circles represent actual temperature readings.


TEMPERATURE (°C)
20
25
30
LIGHT
DARK
LIGHT
1500
2100
0300
0900
SAMPLING TIME (HR.)

guaranteed that the erythrocyte fraction was not disturbed. Cotton swabs were used to absorb this plasma layer. The remaining erythrocytes were sealed and stored at -80°C. Plasma and erythrocytes can be stored in this manner for up to one year without significant changes in ionic composition (Smeda, 1978).

The smaller portion of blood was used for hematological determinations (hematocrit, hemoglobin content). Weight and length of the specimens were recorded.

C) Tissue Samples

Epaxial muscle was taken from each side below the dorsal fin. These were dissected free from any obvious skin, scales, fat, bone and connective tissue. After light blotting on absorbent tissue, tissues were double-wrapped in parafilm and stored at -80°C.

3. <u>Tissue Preparation for Analysis</u>

Frozen tissues were thawed and placed in clean, dry test tubes of known weight and wet tissue weights obtained. Water content weight (g $H_2O \cdot kg^{-1}$; wet weight $\simeq$ ml kg^{-1}) was determined by diference, following dehydration for 24 hrs at 105°C.

Extractions were carried out by the method of Little (1964). This involved extraction of dried samples for 24 hrs, with periodic shaking, in 5.0 ml of 0.1 N HNO_3 as it allowed for the analysis of all ions on each tissue sample.

4. Hematological Determinations

A) Hematocrit (Packed Cell Volume, PCV).

Hematocrit values were determined in triplicate on freshly drawn whole blood samples, using Fisher micro-hematocrit tubes centrifuged at 13,000 RCF (relative centrifugal force) for 5 min in an Adams microhematocrit centrifuge. Packed cell volumes were determined utilizing an Adams microhematocrit reader.

B) Hemoglobin Determination

Hemoglobin determinations were performed in duplicate using the alkaline hematin method (Anthony, 1961). 20 µl aliquots of fresh whole blood were pipetted into 5.0 ml of 0.1 N NaOH, as were 20 µl, 10 µl, and 5 µl , volumes of Hematrol (Clinton Laboratories, Santa Monica, Ca.) with 18.5 g/100 ml stabilized human hemoglobin. Both samples and standards were placed into a boiling water bath for 5 min, allowed to cool and absorbances read at 560 nm on a Bausch and Lomb Spectronic 100 spectrophotometer. Molar concentrations of hemoglobin per litre of blood were calculated assuming molecular weight of 68,000 g Hb/mole (Prosser, 1973). Molar concentrations of hemoglobin per litre of packed erythrocytes were also estimated, using hematocrit values corrected for "trapped plasma" (see later).

5. Cation Determinations

Because of the small volume of sample obtained, cation determinations had to be made on single dilutions. Distilled water was used in making up all solutions.

A) Blood

(i) Erythrocytes

In general, 100 µl of packed erythrocytes were pipetted into 5.0 ml of distilled water. However, in cases of particularly small sample, 20 µl volumes were pipetted into 5.0 ml of distilled water. These were vortexed and allowed to stand for at least 1 hr at 2.0°C. In the instance of 20 µl samples, 5.0 ml of 15.2444 g $SrCl_2 \cdot 6 H_2O$/1 H_2O was added by pipette, whereas 5.0 ml volumes of 15.3662 g $SrCl_2 \cdot 6 H_2O$/1 H_2O were added for the 100 µl samples. After vortexing a second time, cell debris was removed by centrifugation (IEC Clinical Centrifuge, Damon I.E.C. Division, Needham Hts., Mass.) and the clear supernatant obtained, transferred to separate test tubes and refrigerated (2.0°C) prior to analysis. All determinations were done on the same day to avoid refreezing and thawing.

This method has been found to yield satisfactory results for cation determinations by Smeda (1978), who also noted that nitric acid digestion (Little, 1964; Houston &

Mearow, 1978), a harsher method of extraction did not yield more complete extractions, or more consistent results.

(ii) Plasma

100 µl of thawed plasma were pipetted into 10.0 ml of 7.6831 g $SrCl_2 \cdot 6\ H_2O$/1 H_2O, mixed thoroughly and held at 2.0°C prior to analysis.

B) Tissue

500 µl of tissue extract was pipetted into 10.0 ml of 7.9874 g $SrCl \cdot 6H_2O$/1 H_2O. Prior to analysis, samples were mixed and held at 2.0°C.

C) Standards

BDH single element atomic absorption standards (BDH Chemicals, Toronto, Ontario) were utilized to construct calibration curves from which sample concentrations could be determined. These contained the same levels of $SrCl_2$ as samples . Standards bracketed the range of sample concentrations encountered; each standard series ranging from a minimum of 5 to a maximum of 9 concentrations.

All standards included all 4 elements analyzed, to compensate for interference between ions. Ramirez-Monoz (1968) as well as Byrne, et al. (1972) have noted that determinations of K^+ with excess Na^+ present result in higher K^+ values than are found if no Na^+ is present. Because considerable amounts of K^+ and Na^+ are found in all blood fractions, the Na^+ in the blood causes positive interference and results in higher optical density readings for K^+. The reverse effect, that is K^+ on Na^+, has not been shown to occur.

D) Determinations

Cation electrolyte values were obtained using either Unicam SP-90 and a Perkin-Elmer 372 atomic absorption spectrophotometer. In the case of the SP-90, K^+ and Na^+ were analyzed on the emission mode, as this produces less cross-interference, better sensitivity and near-linear calibration curves (Smeda, 1978). The absorption mode was utilized for the determination of Mg^{2+} and Ca^{2+} because of the better resolution obtained (Smeda, 1978). The settings employed in the operation of the Unicam SP-90 are listed in the following Table.

TABLE 6 : Atomic Absorption Spectrophotometer Settings

Mode	Ion	Wave-length (mμ)	Slit width (nm)	Burner height (cm)	Air flow l/min	Fuel flow cc/min	Lamp current (mA)
Emission	Na^+	589.0	0.08	2.0	5	1000	0
	K^+	766.5	0.10	2.0	5	1000	0
Atomic Absorption	Mg^{+2}	422.7	0.10	1.0	5	1000	12
	Ca^{+2}	285.2	0.08	1.0	5	1200	12

Optical density values were recorded on a Fisher Recordall Series 5000 strip-chart recorder (Fisher Scientific Co., Don Mills, Ontario).

The Perkin-Elmer 372 was used in the same manner as the Unicam SP-90, except in the case of Ca^{2+}. This ion was analyzed on the Emission mode as it was more sensitive to the Ca^{2+} concentrations encountered, and also provided finer control over gain settings. The following settings were used in the operation of the Perkin-Elmer 372.

TABLE 7 : Atomic Absorption Spectrophotometer Settings

Mode	Ion	Wave-length (mμ)	Slit width (nm)	Air flow (l/min)	Fuel flow (cc/min)	Lamp current (mA)
Atomic Absorption	Mg^{2+}	285	0.7	55	32	15
	Na^{+}	589	0.7	55	32	-
Emission	K^{+}	766	0.7	55	32	-
	Ca^{2+}	423	0.7	55	32	-

It should be noted that values on one instrument were checked on the other in order to ensure the consistency of the results. The Unicam SP-90 was used during the initial portion of the investigation, but was later replaced with the Perkin-Elmer 372.

During all determinations, the instrument was blanked with a solution of 7.607 g $SrCl_2 \cdot 6H_2O$/1 H_2O. This was done initially as well as between samples. For the appropriate ion, a standard series was obtained between each group of 10 samples in order to check for drift.

Concentrations of plasma and erythrocyte samples were determined from the calibration curve. Values for the latter were corrected for trapped plasma (see later). Tissue cations were calculated using the following formulae:

$$\text{Xppm/Kg} = \frac{(\text{Xppm})\ (\text{dilution Factor})\ (5 + \text{T})}{(\text{T})}$$

$$\text{XmM/Kg} = \frac{\text{ppm/Kg}}{\text{X molecular weight}}$$

where Xppm = parts per million values estimated from the calibration curve for the appropriate cation

T = tissue wet weight in Kg

6. Chloride Determinations

All determinations of Cl^- were made using a Buchler-Cotlove Chloridometer (Buchler Instruments Inc., Fort Lee N.J.).

A) Blood Preparation

Plasma, packed erythrocytes, blanks and standards were prepared in duplicate. 10 µl volumes of each were pipetted into vials containing 2.0 ml of chloride reagent (0.1 N HNO_3 + 10% acetic acid solution). The standard utilized was Versatol (General Diagnostics, Morris Plains, N.J.), a standardized human reference serum containing 103 mM/l Cl^-. Blanks consisted of distilled water plus chloride reagent. Plasma chlorides were analyzed immediately whereas erythrocytic samples were digested for 12 hrs before determination. Standards employed in erythrocytic determinations were also allowed to stand for 12 hours. Just prior to analysis, 2 drops of gelatin reagent (6.2 g of a reagent containing 60:1:1 gelatin:thymol blue:thymol/l H_2O, prepared by Buchler Instruments Inc.) were added to each vial.

B) Tissue Preparation

Tissue extracts, blanks and standards were prepared, in duplicate, by pipetting 1.0 ml volumes into 2.0 ml of chloride reagent. A solution containing 4 µmoles NaCl/ml distilled H_2O was used as the tissue standard. Blanks consisted of a solution of 0.1N HNO_3. 2 drops of gelatin reagent were added to each vial immediately prior to analysis.

C) Determinations

The operation of this instrument is based on the release of Ag^{2+} ions into solution with the simultaneous measurement of solution conductivity. The Ag^{2+} released, binds to the Cl^- in solution to form $AgCl_2$. When the end point is reached, all Cl^- is in the form of $AgCl_2$, free Ag^{2+} accumulates and the solution conductivity rises. The Ag^{2+} is released into the solution at a constant rate so that the time taken to reach the end point is linearly proportional to the Cl^- concentration. In this investigation, the end point solution conductivity of the titration is achieved when a value of 10.0 mA is obtained.

Blanks were first run to condition the Ag^{2+} electrode and obtain stable blank time readings. Standards, followed by 10 samples were then processed and titration times recorded. Plasma and erythrocytic chloride concentrations were obtained using the following formula:

$$\text{Sample Concentration (mM/l)} = \frac{Ts - Tb}{T_{STD} - Tb} \times [Std]$$

Tissue chlorides were determined from the following formula:

$$\text{Sample Concentration (mM/Kg)} = \frac{(Ts - Tb) \cdot [Std]}{(T_{STD} - Tb) \cdot (t_{wt})}$$

where Ts = titration time of sample (sec)

Tb = titration time of blank (sec)

T_{STD} = titration time of standard (sec)

[Std] = concentration of standard

t_{wt} = tissue wet weight in gm

Erythrocytic values were corrected for "trapped plasma" (see later).

7. Water Determinations

The determination of tissue water content has already been covered under the topic of Tissue Preparation for Analysis. The volume of plasma or packed erythrocytes utilized was 10 µl. These samples were first weighed and then dried for 24 hours at 70°C, followed by 48 hours at 103°C. From this, water content was calculated and expressed in two ways : 1) as a percentage of the total weight and 2) the volume of H_2O occupying a volume of plasma or erythrocytes. The volume of H_2O in erythrocytes was corrected for that contributed by trapped plasma.

8. <u>Trapped Plasma Factor</u>

Errors are incurred in cation and chloride determinations on packed erythrocytes due to plasma trapped within the interstices of cells. To compensate for this, the percent volume of plasma occupying an erythrocytic column was used. A value of 2.82%, as determined by Smeda (1978), was utilized. This value was found to be in agreement with the value of 3.0% determined by Catlett & Millich (1976) for goldfish. The following equation was used to determine the electrolyte levels for a measured volume of erythrocytes.

$$\text{Correct level of electrolyte (mM/l cells} = \frac{(E)-(P \cdot 0.282)}{0.9718}$$

where E = mM of electrolyte/l of non-corrected packed cells

and P = mM of electrolyte/l of plasma

9. Statistical Analyses

All statistical analyses were carried out with a Wang 2200 desk top computer (Wang Laboratories Inc., Tewksbury, Mass.) equipped with a line printer. Statistical analyses were performed using automatic or manually run programs developed within the Department (by Mr. P. Steele).

Descriptive statistics including mean, variance, standard deviation, standard error and the 95% confidence interval of the mean were first obtained. Comparisons were then undertaken utilizing single-classification analysis of variance. If required, data were subjected to arc-sine transformation before analysis. Significance was attributed to differences at the $P < 0.05$ and $P < 0.01$ levels.

The least squares method was employed when linear and geometric regression analyses were performed. Coefficients of correlation for the regressions were analyzed to estimate the significance of the fits obtained. In this case, significance was attributed only to fits at the $P < 0.01$ or $P < 0.05$ level.

RESULTS

The structure of the study was such that diurnal variations in the different values could be examined as well as the more obvious effects of constant and cycling temperatures. This has been done in each of the following sections. The data locations are noted below. For purposes of presentation, Figures 12 through 20 have been used to summarize the main findings. In all cases, values are given as mean $\pm$ 1 standard error of the mean, with a significance summary which indicates variation with time for each temperature treatment, and differences between the thermal acclimation groups at specific sampling times. In these summaries, absence of significance is indicated by underlining. Consequently, all other comparisons indicate differences significant at the 0.05 level or better.

(A) Location of Data

Appendix Tables 10 through 33 contain all the original data gathered in the investigation. Summarized accounts are presented in Appendix Tables 1 to 9. In order to illustrate general trends, data are in Figures 12 to 20 within the text.

(B) Hematological Parameters

(1) Hemoglobin Content (Figure 12A)

The most noteworthy observations may be summarized as follows:

(a) Animals maintained at constant temperature were characterized by marked diurnal variations in blood hemoglobin content. These, however, exhibited no consistent pattern. In the 20°C group, for example, maximum levels were observed at 15:00 and 21:00 h. By contrast, those acclimated to 25° and 30°C tended to have higher concentrations at 03:00 than at other periods.

(b) At specific sampling intervals, hemoglobin did not vary with temperature in a consistent fashion.

(c) Among the cycled animals, hemoglobin levels were, in general, higher than those of constant temperature fish and relatively stable.

(2) Packed Cell Volume (Figure 12B)

Packed cell volume variations, not surprisingly, tended to resemble those in hemoglobin content. No significant variation with time was seen in the cycled animals, and PCV values tended to be higher than those seen in constant temperature groups. Again, no consistent trends were apparent in relation to either temperature or time of sampling in the

Figures 12 : Hematological parameters of goldfish acclimated to 20°C (open circles), 25°C (open squares), 30°C (open triangles)

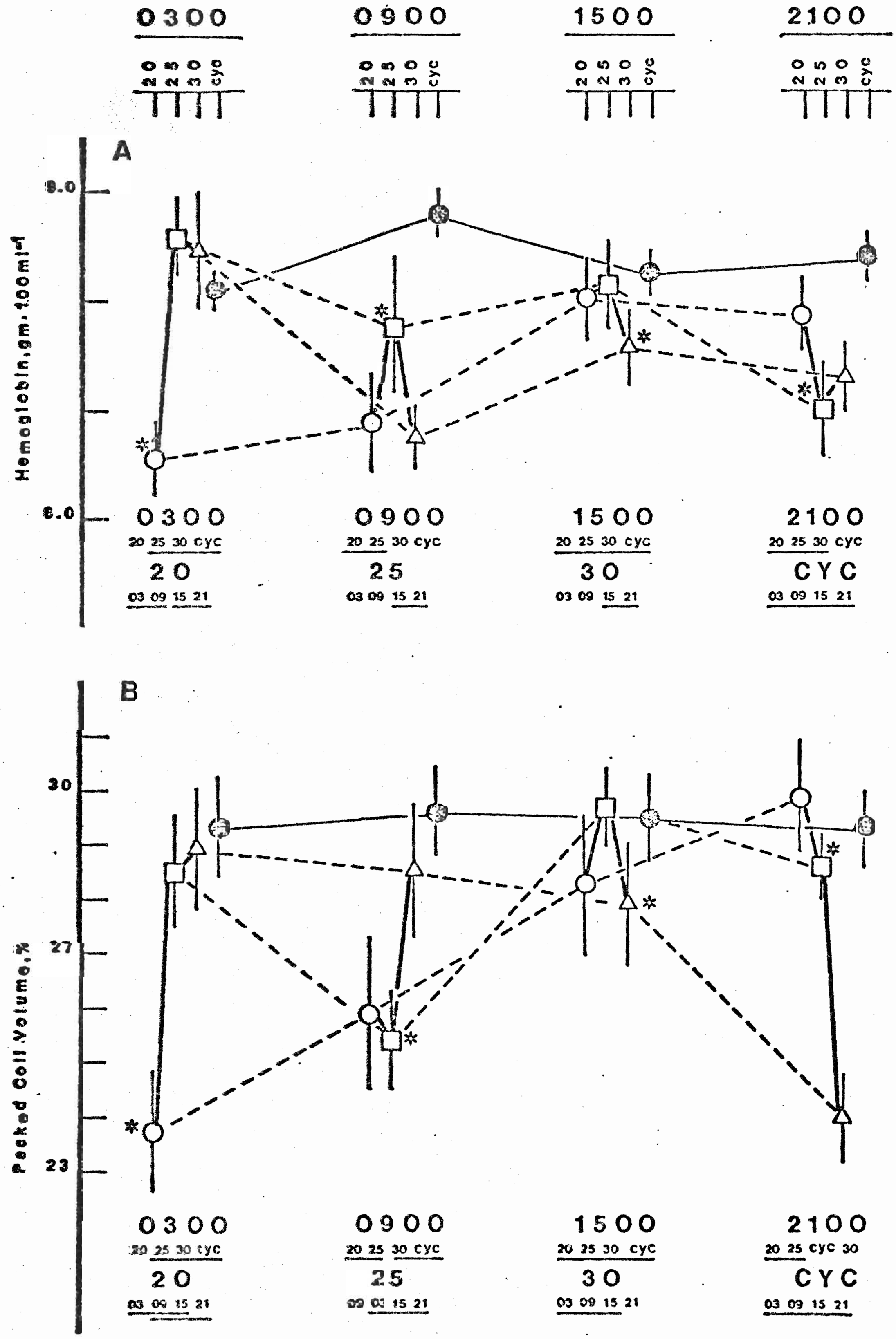
A & B

and a cycling temperature of 25° ± 5°C (closed circles) at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A) Hemoglobin content ($gm \cdot 100\ ml^{-1}$).

B) Packed cell volume (%)

Symbols are centered on the mean with vertical bars representing 1 standard error of the mean. Stars denote static temperature values equivalent in time and temperature to the cycling thermal regime. Heavy lines connect the static temperature readings from the lowest to highest temperatures. Time and temperature significance summaries are located at the bottom of each figure with the absence of significance indicated by underlining.


0300
0900
1500
2100
20 25 30 cyc
A
9.0
6.0
Hemoglobin, gm·100ml⁻¹
0300
20 25 30 cyc
20
03 09 15 21
0900
20 25 30 cyc
25
03 09 15 21
1500
20 25 30 cyc
30
03 09 15 21
2100
20 25 30 cyc
CYC
03 09 15 21
B
30
27
23
Packed Cell Volume, %
0300
20 25 30 cyc
20
03 09 15 21
0900
20 25 30 cyc
25
09 03 15 21
1500
20 25 30 cyc
30
03 09 15 21
2100
20 25 cyc 30
CYC
03 09 15 21

latter animals.

(3) Correlation of Hematological Parameters with Specimen Weight

Since oxygen consumption in goldfish is weight-specific in nature, increases in the blood oxygen capacity would be advantageous to smaller animals within the restrictions that are imposed by concommitant increases in blood viscosity and the effects of this upon cardiac work requirements. Accordingly, a correlation analysis was carried out, and the results of this are summarized in Appendix Tables 34 and 35. Such significant correlations as were encountered tended to occur at the lower constant temperatures, and were a consistent feature of the hematological status of the goldfish.

(4) Summary of Hematological Findings

Under cycling temperature conditions, both hemoglobin and packed cell volume tended to be higher and more stable than was the case with animals maintained at constant temperatures. There was little evidence of weight-specific variation in either parameter.

(C) <u>Plasma Water-Electrolyte Status</u>

(1) Principal Ions : Sodium and Chloride (Figures 13A and E).

Plasma sodium was characterized by three principal features. While significant diurnal changes were encountered, they were, in general, of modest magnitude (maximum: $\sim 5\%$) in both the constant and cycling temperature groups, and followed no consistent pattern. Among animals acclimated to constant temperature conditions, sodium concentrations tended to vary inversely with temperature, the principal exception to this being seen at 03:00 h. Under cycling temperature conditions, sodium levels were well below those of the constant temperature groups. This became particularly notable when the cycled animals were compared to those at constant temperature equivalent to the particular times at which the former were sampled.

As with sodium, chloride levels also exhibited only minor diurnal variations, with the maximum mean concentration differences amounting to only 2-3 $mmol \cdot l^{-1}$. Cycled animals were also characterized by substantially lower chloride concentrations than the constant temperature groups, with the differences being quite pronounced and highly significant when time and temperature-equivalent comparisons were considered. By contrast with sodium, however, chloride levels in constant-temperature goldfish increased with increase in temperature.

(2) Minor Ions : Potassium, Calcium, Magnesium (Figure 13B, C and D).

Diurnal variations in plasma potassium concentration were significant at the lower constant temperatures (20°, 25°C), but not at 30°C or in the cycled animals. In the former instance, the most notable distinction was the higher values seen at 15:00 and 21:00 hr. No consistent pattern of variation in potassium content with temperature was apparent in the constant-temperature fish. As had been the case with sodium and chloride cycled animals exhibited lower potassium levels than did those acclimated to steady conditions. The differences observed were not, however, marked, and were apparently non-significant for the most part.

Calcium concentrations varied to some extent on a diurnal basis. Significant differences were present, but no consistent pattern of diurnal change was obvious. This was also true with respect to thermally-related changes in the constant temperature groups. Unlike sodium, chloride and potassium, calcium in cycled animals was not markedly distinct from that in constant-temperature fish. Similar comments apply to magnesium as well. No consistent pattern of diurnal-variation was apparent. Cycled and constant-temperature animals were not distinct in terms of this, or actual concentrations. Variation in magnesium levels in the three constant temperature groups were consistent only in the occurrence of minima at 25°C relative to 20° and 30°C.

(3) Plasma Water (Figure 13F)

In most respects, alterations in plasma water under the

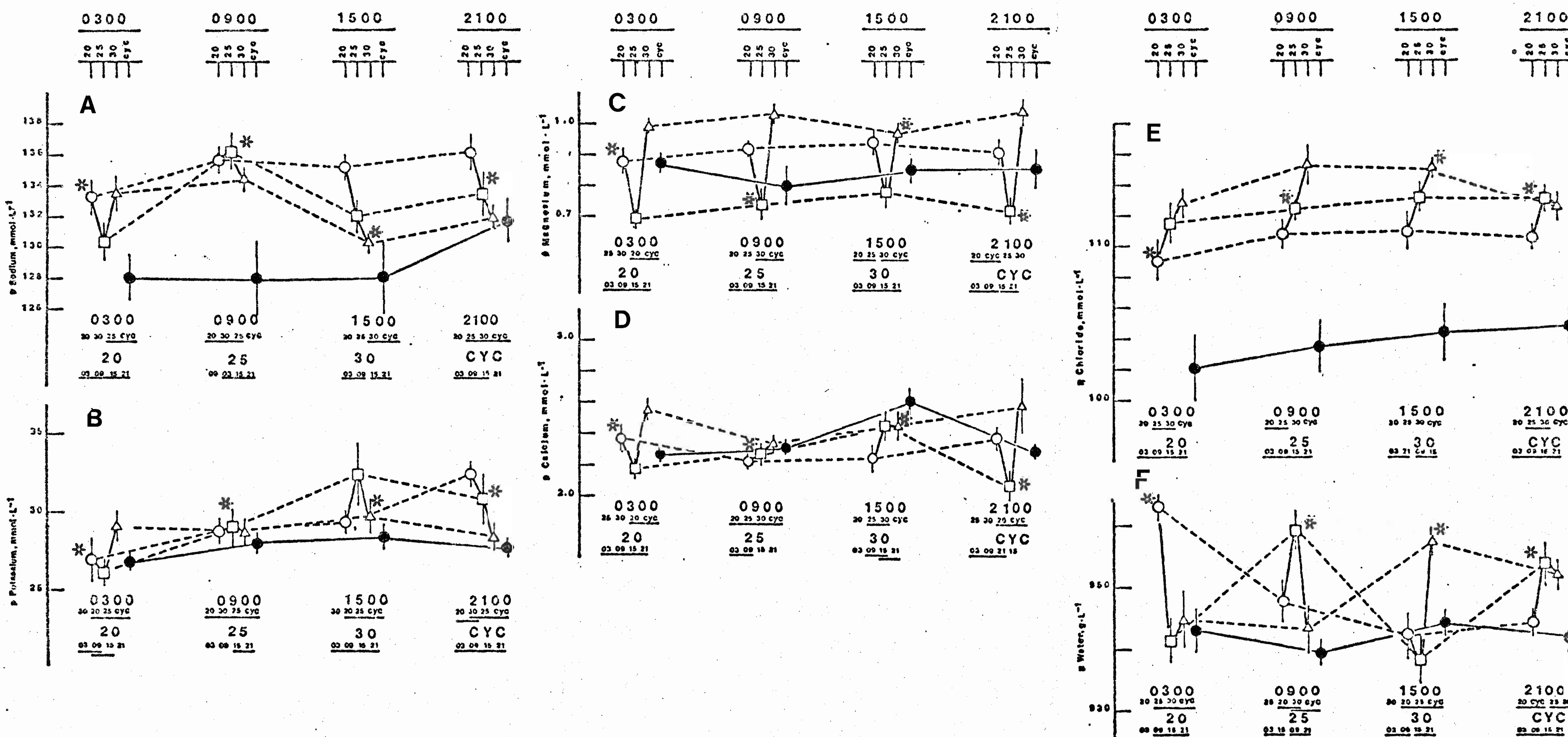
Figures 13 : Plasma water ($g \cdot l^{-1}$) and electrolyte ($mmol \cdot l^{-1}$) levels of goldfish acclimated to 20°C, 25°C, 30°C and a diurnal cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A to F

A) Sodium B) Potassium C) Magnesium

D) Calcium E) Chloride F) Water content

Symbols as in Figures 12, A and B



A
B
C
D
E
F
p Sodium, mmol·L-1
p Potassium, mmol·L-1
p Magnesium, mmol·L-1
p Calcium, mmol·L-1
p Chloride, mmol·L-1
p Water, g·L-1
0300
0900
1500
2100
20
25
30
CYC

conditions imposed were similar to those noted for electrolytes. Thus, diurnal variations in the constant temperature groups were seen but did not follow a consistent pattern. This was true as well of the relationship between plasma water and temperature in these animals. The cycled specimens exhibited considerably less diurnal variation. They generally had lower water concentrations than did those at constant temperature, and this was particularly apparent when the cycled group was compared to groups at equivalent temperature during comparable sampling periods.

In summary, three points emerged from this feature of the investigation:

(i) Goldfish maintained under a cycling temperature regimen tended to exhibit relatively less diurnal variation in plasma composition than did animals held under constant temperature conditions. Calcium ion was, perhaps, the principal exception to this generality.

(ii) In cycled animals, the principal plasma ions, chloride and sodium, were maintained at levels well below those seen in the constant temperature groups. This was particularly apparent when comparisons were made with animals at the same but constant temperature when sampled. Much the same was true of potassium and water. By contrast, calcium and magnesium levels were not notably different in the cycled and constant temperature groups. In general, those of the cycled fish tended to be most similar to the levels seen in animals at the same sampling temperature.

(iii) Only sodium and chloride exhibited consistent or near-consistent variation in concentration with temperature in the constant groups; sodium tending to vary inversely while chloride increased with increasing temperature.

(D) Erythrocytic Water-Electrolyte Status

(1) Sodium and Potassium (Figures 14A and B, 15A and B)

Similar trends in sodium levels were noted regardless of whether values were expressed in terms of packed cells or cell water. Diurnal variation was restricted to the 25°C and 25° ± 5°C thermal regimes. No consistent trend could be detected for 25°C statically-acclimated animals while cycled temperature specimens were characterized by an unusually low 03:00 hr value. A relationship could be distinguished between the cycling temperature concentrations and those of static temperatures paralleling the actual cycle, with the former surpassing the latter. Among the static temperature groups, there was a significant increase in sodium level at higher temperature at all but 09:00 hr.

Although differences in potassium concentrations dependent on manner of expression, i.e., packed cells or litre cell water, were encountered, these led to only minor variations in significance and the general trends exhibited. Diurnal variation in the potassium levels of constant temperature animals was such that no consistent patterns emerged. Concentrations under the cycling thermal regime, however, tended to decrease during the increasing phase of the cycle.

When static temperature levels corresponding to the temperature cycle were compared, they were found to be lower than those obtained under the cycle itself. This was not true when static temperature values, as a whole, were employed. The ordering of K^+ concentrations in statically-acclimated fish was 20°C, 30°C and 25°C (highest to lowest). The only case in which the pattern differed significantly from this order was at 21:00 hr for values expressed as mmol l^{-1},cell H_2O.

(2) Magnesium and Calcium (Figures 14C and D, 15C and D)

As was the case with potassium, the mode of expression influenced the trends observed but, again, the differences were not great. Both Mg^{2+} and Ca^{2+} were characterized by diurnal stability. Only two notable features could be distinguished: 1) cycled temperature Ca^{2+} concentrations were significantly reduced at 15:00 hr; 2) at 03:00 hr Mg^{2+} levels of 25°C statically-acclimated fish were elevated. The most striking feature of temperature-related alteration in Mg^{2+} and Ca^{2+} was the inverse nature of concentration in relation to acclimation temperature.

In general, Mg^{2+} levels in cycling animals were intermediate to those of statically-acclimated animals, while Ca^{2+} concentrations were very similar to constant 30°C values. However, when compared to static temperature readings paralleling those of the cycle, Mg^{2+} levels tended to be above, and Ca^{2+} concentrations below those of the appropriate groups.

(3) Chloride (Figures 14E and 15E)

In terms of manner of expression of concentrations, chloride exhibited many of the features seen with potassium, magnesium and calcium. Of the four thermal regimes employed, only the cycling temperature condition produced diurnally stable values. Although significant differences were not always obtained, static temperatures were characterized by a consistently low 03:00 hr Cl^- concentration.

With the exception of the 03:00 hr period, cycling temperature concentrations were below those obtained under constant conditions. This was true of comparisons involving

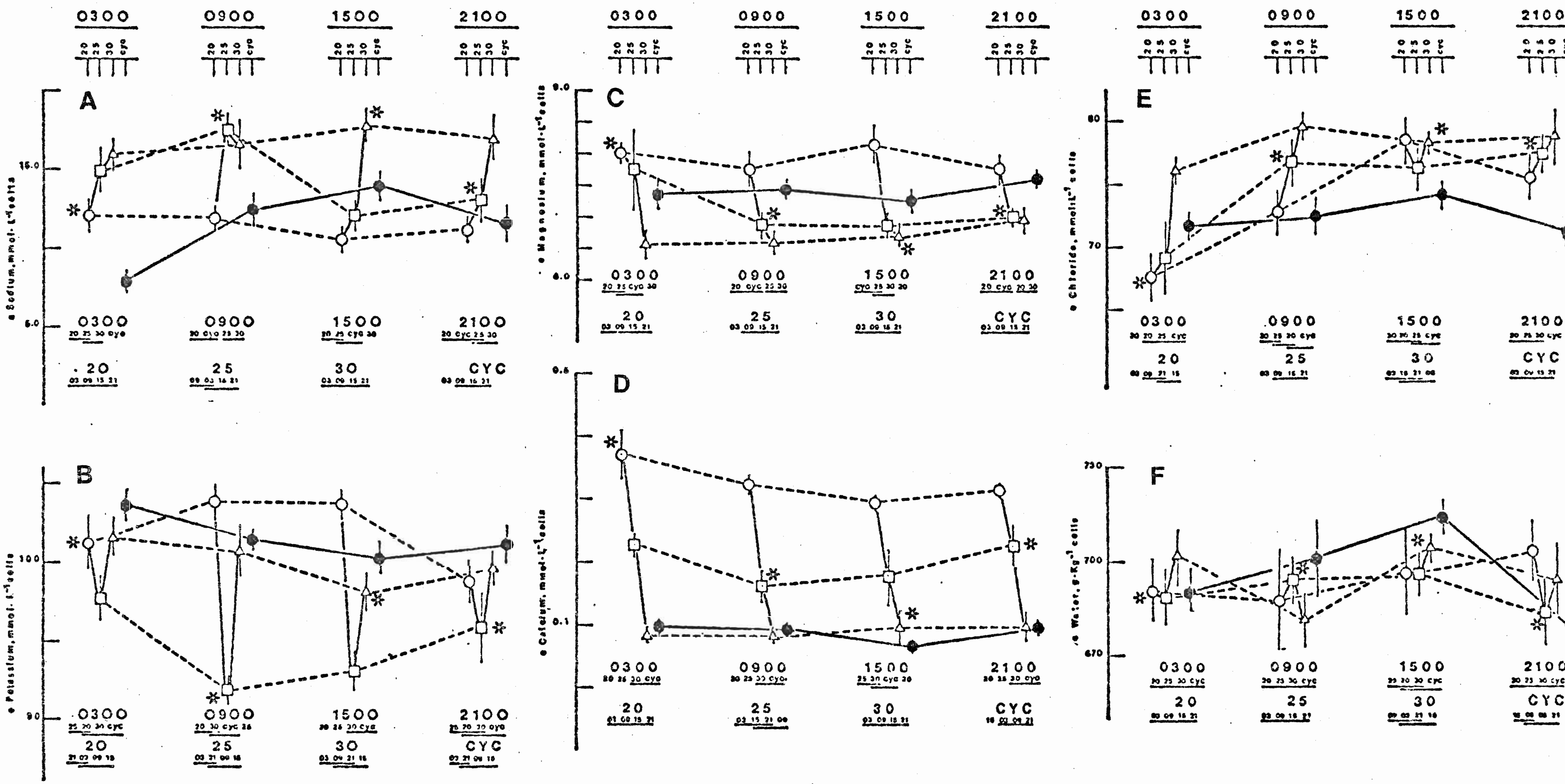
Figures 14 A to F

Erythrocytic water ($g \cdot Kg^{-1}$, packed cells) and electrolyte ($mmol \cdot l^{-1}$, packed cells) levels of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00, 15:00 hr and 21:00 hr.

A) Sodium B) Potassium C) Magnesium

D) Calcium E) Chloride F) Water content

Symbols as in Figures 12, A and B



A
B
C
D
E
F
0300
0900
1500
2100
20
25
30
CYC
15.0
6.0
100
90
9.0
6.0
0.5
0.1
80
70
730
700
670

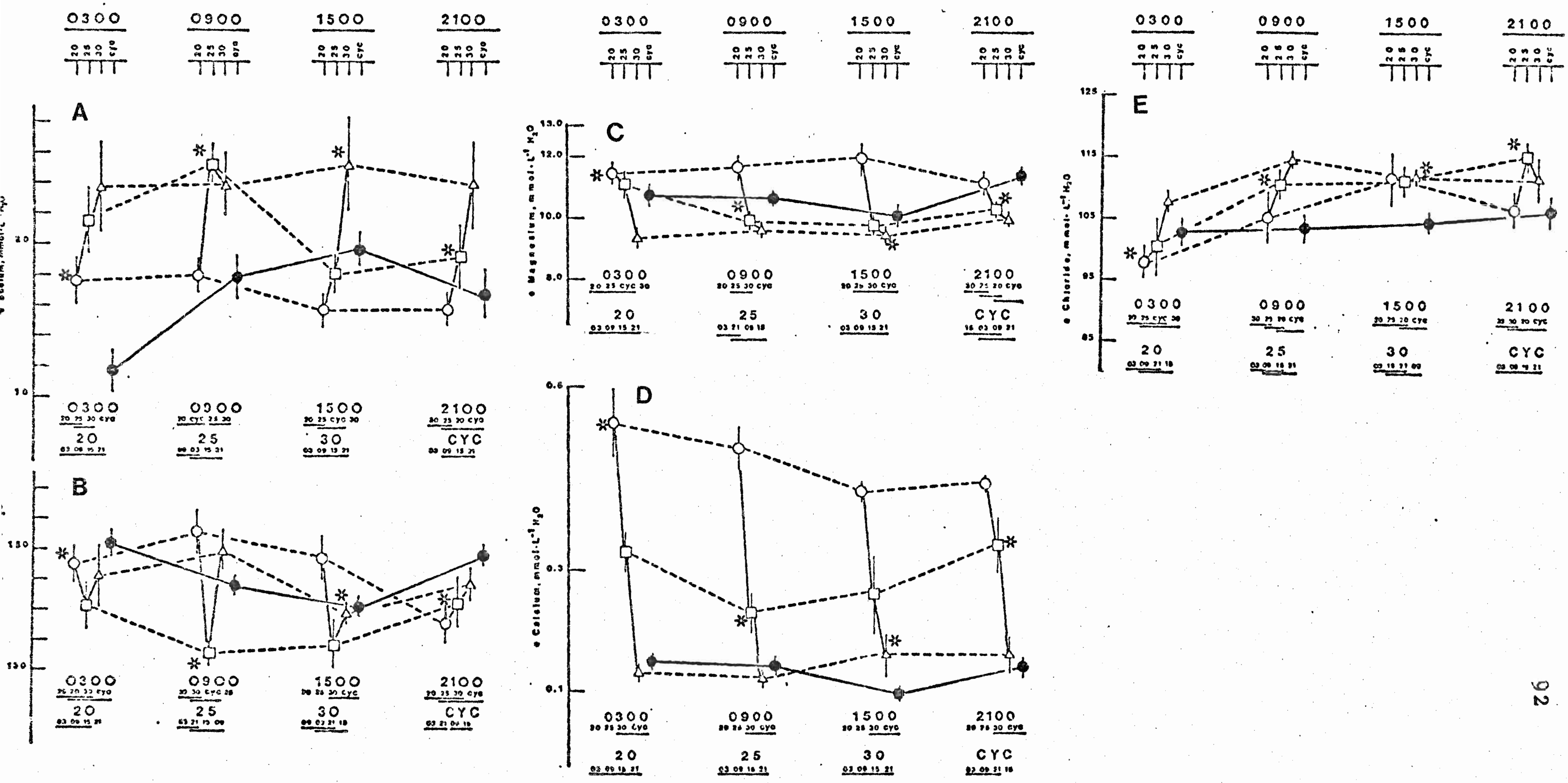
Figures 15 : Erythrocytic electrolyte levels ($mmol \cdot l^{-1}$, cell H_2O) of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of

A to E — 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A) Sodium B) Potassium C) Magnesium

D) Calcium E) Chloride

Symbols as in Figures 12, A and B


A
B
C
D
E
0300
0900
1500
2100
20
25
30
CYC
e Magnesium, mmol·L⁻¹ H₂O
e Calcium, mmol·L⁻¹ H₂O
e Chloride, mmol·L⁻¹ H₂O

static temperature values in general and was pronounced in relation to those equivalent to the temperature cycle. Chloride levels in statically-acclimated fish increased with acclimation temperature. It should be noted, however, that this pattern was characterized by some inconsistencies (15:00 hr for values as $mmol \cdot l^{-1}$, packed cells; 21:00 hr for values as $mmol \cdot l^{-1}$, cell H_2O) and non-significant differences.

(4) Erythrocytic Water (Figure 14 F)

Red cell water content in fish maintained at constant temperatures of 20°C and 25°C did not exhibit diurnal fluctuations. Those at 30°C did not exhibit any consistent pattern. Under the cycling regime, water content increased from 03:00 hr (20°C) to 15:00 hr (30°C) and then fell at 21:00 hr.

Water content did not vary with temperature. Indeed, only one significant variation was reported over the entire sampling period. Thus, no consistent temperature-related variations could be distinguished.

The following points summarize the findings of this portion of the investigation :

1) In general, erythrocytic water and electrolytes exhibited relatively little diurnal variation of a consistent character. Modest, but significant variations were, however, observed in some instances: a) cycling temperatures: Na^+, K^+, Ca^{2+},

water content; b) 25°C : Mg^{2+} and c) 20°C, 25°C and 30°C : Cl^{-}.

2) When static temperature readings paralleling the cycle were considered, Na^{+}, Ca^{2+} and Cl^{-} concentrations in the former exceeded the latter, while the converse was true of K^{+} and Mg^{2+}. When concentrations in the static temperature groups were compared with those of cycling animals, differences could be discerned for Mg^{2+}, Ca^{2+} and Cl^{-}. In the first, values from cycling animals were intermediate in magnitude, in the second, they resembled concentrations at 30°C and, in the last, they were lower than the static temperature levels.

3) Three concentration trends were noted in statically-acclimated animals: a) increases in Na^{+} and Cl^{-} concentrations with temperature; b) decreases in Mg^{2+} and Ca^{2+} with temperature; and c) an ordering from highest to lowest of 20°C, 30°C and 25°C in the case of K^{+}.

4) Erythrocytic water content exhibited no significant differences with temperature in the static temperature groups or between static and cycling temperature animals.

(E) <u>Erythrocytic Ion : Hemoglobin Ratios</u>

(1) Na^{+} : Hb and K^{+} : Hb Ratios (Figures 16A and B).

Diurnal fluctuations in Na^{+} : Hb ratios were encountered under the 25°C and 25°$\pm$ 5°C thermal regimes, the 20°C and

30°C values remaining stable with time. In the former, no obvious diurnal pattern was apparent. However, in cycling animals there was a distinct increase in these ratios with increases in cycle temperature. K^+ : Hb ratios were characterized by a greater degree of diurnal variability with overall stability seen only in the case of 30°C acclimated fish. Although significant differences were obtained for 20°C and 25°C animals, these patterns showed no consistent trends. Under cycling temperature conditions, peak values appeared to occur at both the high and low temperatures of the cycle.

With the exception of the 09:00 hr group, Na^+: Hb ratios increased significantly, with temperature under static accclimation conditions. When compared with static levels, in general, the cycling temperature values of both parameters displayed no discernible pattern. However, when parallel readings based on temperatures of the cycle were utilized, a distinct relationship emerged in each case. For Na^+ : Hb ratios, static temperature values exceeded those in the cycling temperature situation. This pattern was reversed for K^+ : Hb ratios at three of the four sampling periods.

(2) Mg^{2+} : Hb and Ca^{2+} : Hb Ratios (Figures 16C and D)

Diurnal variability was confined to the Ca^{2+} : Hb ratios of the 20°C and cycling temperature groups. In both situations, significant differences were associated with the 15:00 hr value.

In general, both exhibited significant decreases in magnitude with increasing static acclimation temperature. Cycling temperature Mg^{2+} : Hb values were found to be of intermediate magnitude while Ca^{2+} : Hb were similar to the 30°C readings. When static values corresponding to cycle temperatures were considered, a definite relationship was seen with respect to Ca^{2+} : Hb. In this case, the former surpassed the latter over the entire duration of the sampling period. No such clear pattern was observed with Mg^{2+} : Hb. Cycling temperature values did not differ significantly from the corresponding static groups in most cases.

(3) Cl^- : Hb Ratio (Figure 16E)

Under each of the thermal conditions employed, some diurnal flunctuations were encountered. However, the patterns exhibited by all statically-acclimated animals were not consistent in nature. Cycling temperature animals displayed uniform values at all times, with the exception of 15:00 hr. This was associated with the increased temperature of the cycle at that time.

Comparison of static values suggested that at all times other

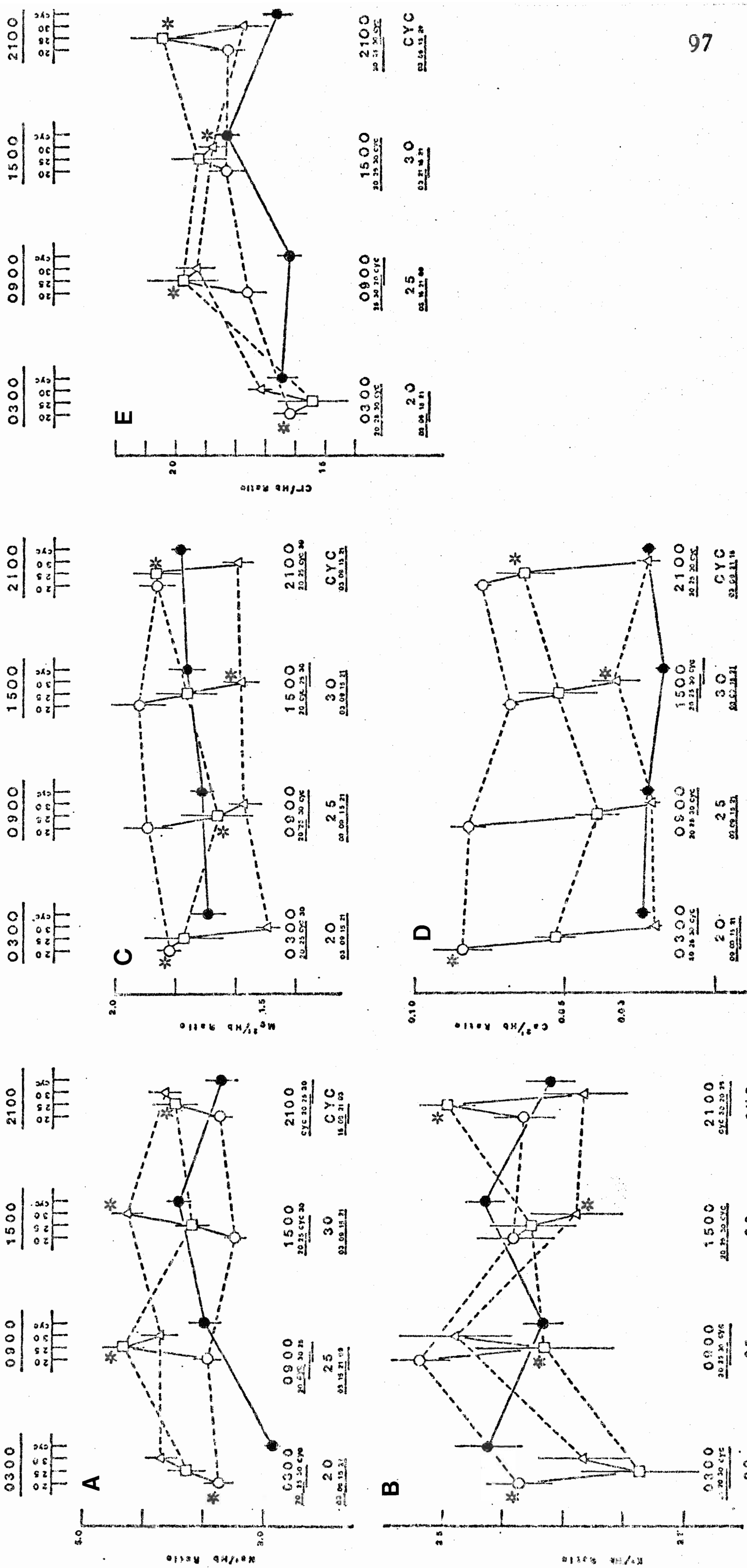
Figures 16 : Ion : hemoglobin ratios (mmol$\cdot$l^{-1}, packed cells/mmol l^{-1}, packed cells) of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A to E

A) Sodium B) Potassium C) Magnesium

D) Calcium E) Chloride

Symbols as in Figures 12, A and B


A
B
C
D
E
0300
0900
1500
2100
20
25
30
CYC

than 03:00 hr, an ordering of the ratios based upon acclimation temperature was present; 25°C values were highest followed by those at 30°C and 20°C. Cycling temperature levels were, for the most part, below those of static animals. This was also apparent when static values corresponding to the temperature cycle were utilized.

To summarize this aspect of the study, the following points can be made:

a) Under cycling temperature conditions goldfish exhibited distinct diurnal patterns in Na^+ : Hb, K^+ : Hb and Cl^- : Hb ratios. The first involved an increase corresponding to increases in the temperature cycle; the second, peak values at both the high and low temperatures of the cycle; and the last, maxima associated with the maximum of the cycle. Although significant fluctuations were also seen under static thermal conditions, no consistent patterns could be distinguished.

b) With the exception of the Cl^- : Hb and Ca^{2+} : Hb ratios, no relationship was apparent in comparison of cycling temperature values and those of static fish. However, comparison of static temperature fish at temperatures and times paralleling those of the cycle, revealed significant trends in all but Mg^{2+} : Hb. Na^+ : Hb, Ca^{2+} : Hb and Cl^- : Hb ratios of statically-acclimated animals exceeded those of the cycling temperature specimens. This was reversed in the case of K^+ : Hb. Mg^{2+} : Hb in cycling temperature animals were intermediate to those of the static temperature situations.

c) Comparisons between static temperature values revealed distinguishable trends in all but K^+ : Hb. Mg^{2+} : Hb and Ca^{2+} : Hb displayed decreases with increasing temperature. The converse was true of Na^+ : Hb readings. The order from highest to lowest Cl^- : Hb ratio was 25°C, 30°C and 20°C.

(F) <u>Muscle Water-Electrolyte Status</u>

(1) Potassium (Figure 17B).

Potassium concentrations in statically-acclimated fish were diurnally stable. Those of animals maintained on the cycling regime exhibited diurnal fluctuations. A maximum at 15:00 hr accounted for most of the significant variations observed, and no consistent pattern was distinguishable.

At other than 15:00 hr. values obtained under static temperature conditions exceeded those of the cycling temperature group, regardless of whether comparisons were made with entire constant temperature groups, or with those obtained under thermal conditions equivalent to the cycle. Although differences were not always significant, potassium levels at 25°C usually exceeded those at 20°C and 30°C at all but one sampling period (03:00 hr).

(2) Sodium and Chloride (Figure 17A and E)

Only at 20°C were significant diurnal changes in Na^+ seen. A ~6.6 to ~4.4 mmol•l^{-1} reduction occurred between 03:00 hr and 09:00 hr; thereafter, concentrations remained relatively stable. Although significant differences were noted under the 30°C and cycling temperature regimes, they were limited in each case to one pair of times (09:00 vs 21:00 hr at 30°C, 09:00 vs 15:00 hr at 25° ± 5°C) and exhibited no discernible pattern. Cycling temperatures were associated with somewhat higher values than were observed in the static groups. This was also evident when these were compared to those of static animals at temperatures corresponding to the cycle. In general, an ordering pattern of 25°C, 30°C and 20°C (highest to lowest) was obtained for the statically-acclimated animals.

Chloride concentrations were also diurnally stable with only minor variations (~1.4 - ~1.6 mmol l^{-1}) apparent, notably under the 25°C and 25 ± 5°C thermal regimes. Maximum concentrations were recorded for fish maintained under cycling conditions. This was true of comparisons made with static temperatures and, in general, for values obtained under constant temperatures corresponding to those of the cycle. Concentrations in 20°C fish tended to exceed those at 25°C and 30°C.

(3) Divalent Cations (Mg^{2+} and Ca^{2+}), (Figure 17C and D)

Both Mg^{2+} and Ca^{2+} concentrations were characterized by diurnal variation at 20°C and under cycling temperature conditions. In the case of magnesium, this was due to a maximum at 21:00hr. An unusually high Ca^{2+} concentration at 03:00 hr accounted for that at 20°C. The cycling temperature group exhibited high Ca^{2+} values at 15:00 hr.

In general, lowest concentrations were observed under cycling temperature conditions, while maximum values in the constant temperature groups occurred at 25°C. In all but one case (20°C Mg^{2+} at 03:00 hr), concentrations in cycled animals were lower than those seen at static temperatures corresponding to the cycle. Magnesium and calcium concentrations of 30°C fish tended to be above those of 20°C specimens.

(4) Muscle Water (Figure 17F)

Diurnal variations were evident in all constant temperature groups other than 30°C. No consistent pattern was noted at 25°C, or in the cycled fishes, while 20°C animals exhibited a high value at 03:00 hr. When compared to static temperature animals, those under cycling temperature conditions displayed no consistent relationship. However, when the comparison was made using static values corresponding to the temperature cycle, cycling temperature concentrations

Figures 17 : Muscle water ($g \cdot Kg^{-1}$) and electrolyte ($mmol \cdot Kg^{-1}$) levels of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A to F

A) Sodium B) Potassium C) Magnesium

D) Calcium E) Chloride F) Water content

Symbols as in Figures 12, A and B

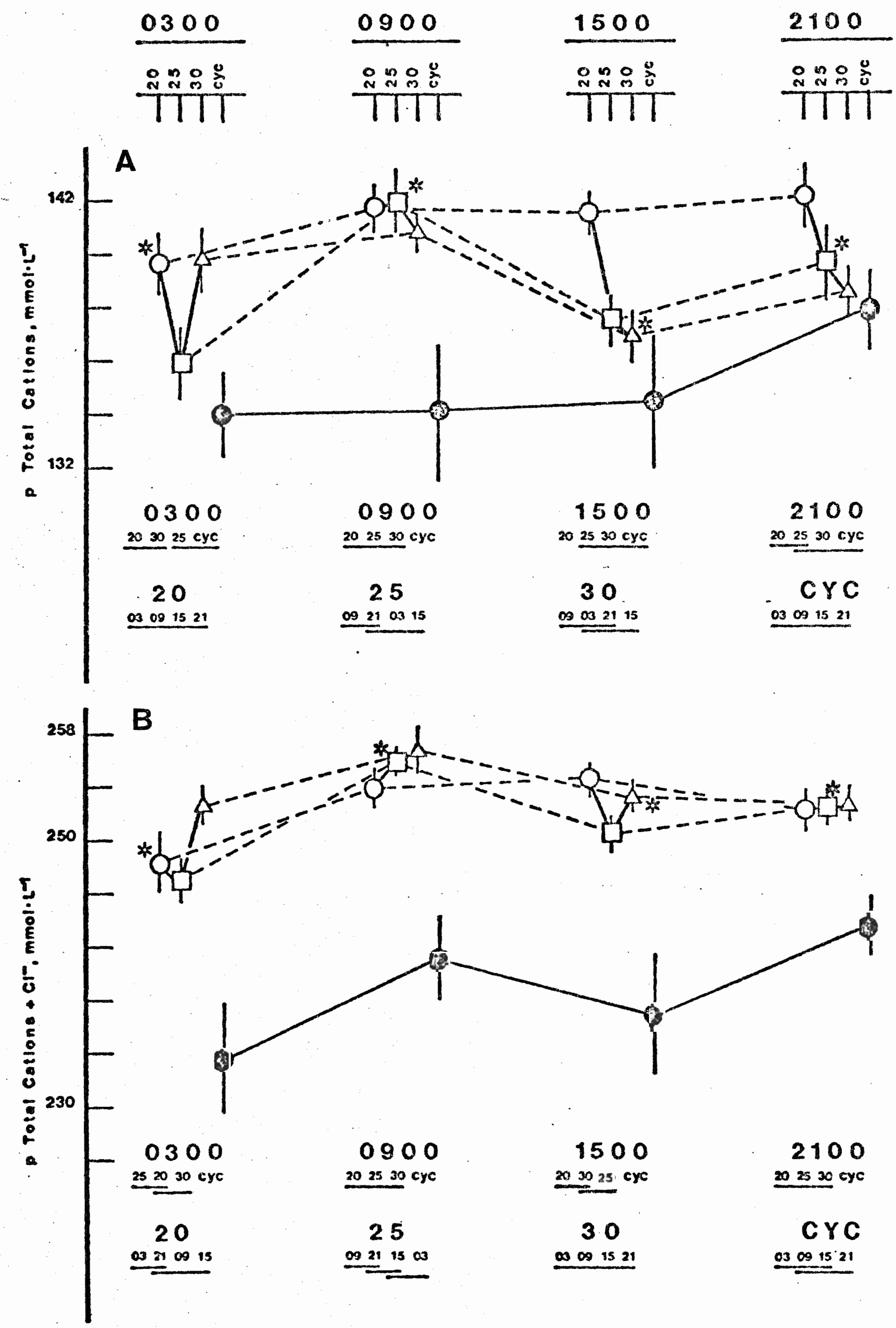
Figures 18 : Plasma total cations (mmol$\cdot l^{-1}$) and total cations + Cl^- (mmol$\cdot l^{-1}$) levels of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° $\pm$ 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A & B

A) Total Cations

B) Total Cations + Cl^-

Symbols as in Figures 12, A and B


0300
0900
1500
2100
20 25 30 cyc
A
142
132
p Total Cations, mmol·L⁻¹
0300
20 30 25 cyc
0900
20 25 30 cyc
1500
20 25 30 cyc
2100
20 25 30 cyc
20
03 09 15 21
25
09 21 03 15
30
09 03 21 15
CYC
03 09 15 21
B
258
250
230
p Total Cations + Cl⁻, mmol·L⁻¹
0300
25 20 30 cyc
0900
20 25 30 cyc
1500
20 30 25 cyc
2100
20 25 30 cyc
20
03 21 09 15
25
09 21 15 03
30
03 09 15 21
CYC
03 09 15 21

temperature animals were lower than the readings of the static thermal regimes, be they overall static values or those paralleling the cycle. This difference was more pronounced in the case of the latter parameter. Static temperature total cation levels tended to decrease with acclimation temperature while total cations + Cl^- were similar under all static temperatures. Both, however, were characterized by inconsistencies, the former primarily at 03:00 hr, the latter at 15:00 hr.

(2) Erythrocyte (Figure 19A and B)

With the exception of 30°C concentrations, diurnal variation in total cation levels was limited to one sampling period under each thermal regime. Total cations + Cl^- concentrations exhibited a greater degree of diurnal stability with only one significant variation reported at 30°C. Consistent patterns were encountered only in the case of 25°C total cations and 20°C total cations + Cl^-. The former decreased from 03:00 hr to 15:00 hr and then recovered to a level equivalent to the 03:00 hr period, while the latter was characterized by a peak 15:00 hr reading. As in plasma, both erythrocytic parameters displayed greater diurnal stability under cycling temperature as compared to the static temperature groups.

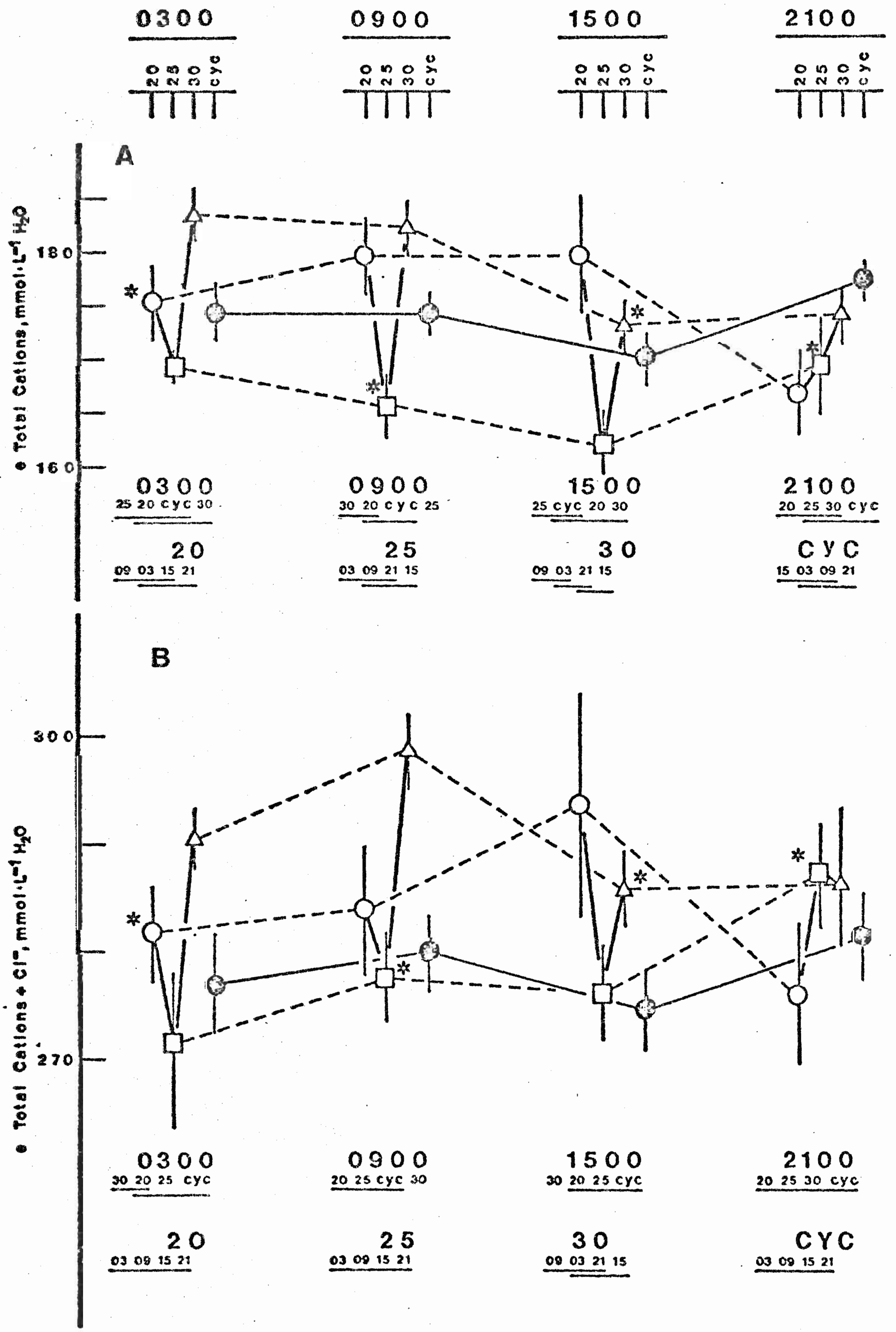
Regardless of the comparison utilized, total cation

Figures 19 : Erythrocytic total cation ($mmol \cdot l^{-1}$, cell H_2O) and total cation + Cl^- ($mmol \cdot l^{-1}$, cell H_2O) levels of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.
A & B

A) Total Cations

B) Total Cations + Cl^-

Symbols as in Figures 12 A and B


0300
20 25 30 cyc
0900
20 25 30 cyc
1500
20 25 30 cyc
2100
20 25 30 cyc
A
• Total Cations, mmol·L⁻¹ H₂O
180
160
0300
25 20 cyc 30
0900
30 20 cyc 25
1500
25 cyc 20 30
2100
20 25 30 cyc
20
09 03 15 21
25
03 09 21 15
30
09 03 21 15
CYC
15 03 09 21
B
• Total Cations + Cl⁻, mmol·L⁻¹ H₂O
300
270
0300
30 20 25 cyc
0900
20 25 cyc 30
1500
30 20 25 cyc
2100
20 25 30 cyc
20
03 09 15 21
25
03 09 15 21
30
09 03 21 15
CYC
03 09 15 21

levels of cycling temperature animals were intermediate to those of the constant temperature groups. Cycling temperature total cation + Cl^- readings were similar to the lower static temperature values but, in general, were less than those of temperatures paralleling the cycle. Although irregularities could be noted (15:00 hr and 21:00 hr), both parameters in the statically-acclimated fish decreased in concentration as follows: 30°C, 20°C and 25°C.

(3) Muscle (Figures 20A and B)

Except for the low 03:00 hr readings reported for 25°C animals, static temperature total cation and total cation + Cl^- levels were characterized by diurnal stability. Cycling temperature readings exhibited a peak associated with the high temperature of the cycle (30°C at 15:00 hr).

In general, the concentrations of both parameters were similar under 20°C and cycling temperature conditions. At all times but the 03:00 hr period, static temperature total cation and total cation + Cl^- levels corresponding to those of the cycle, were greater in concentration than those of the actual cycle. Both parameters of the static temperature groups decreased as follows: 25°C, 30°C and 20°C.

A summarized account of total cations and total cations + Cl^- for all three tissue types can now be presented.

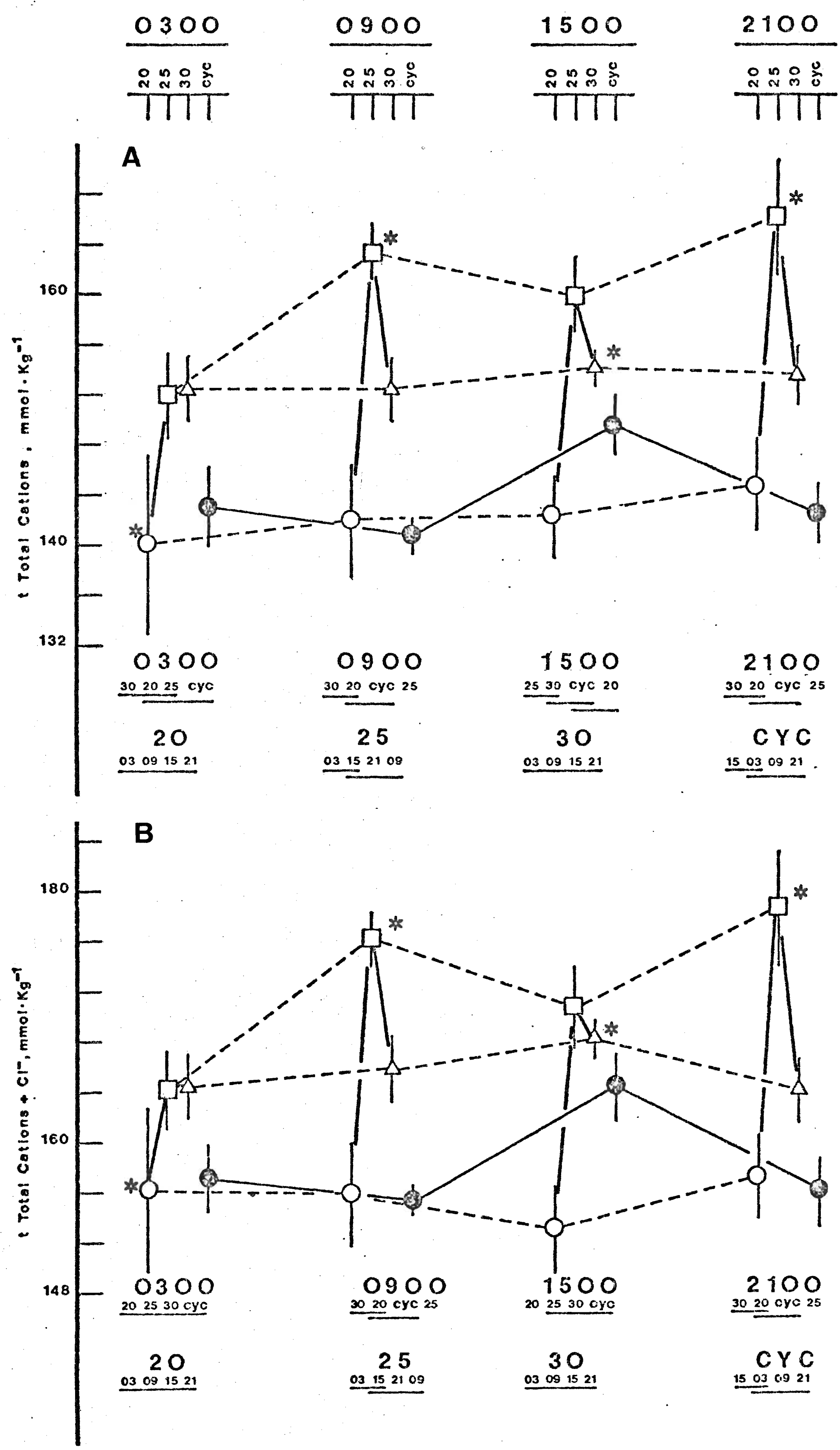
a) Both parameters exhibited a high degree of diurnal

Figures 20 : Muscle total cation ($mmol \cdot Kg^{-1}$) and total
A & B cation + Cl^- ($mmol \cdot Kg^{-1}$) of goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr.

A) Total Cations

B) Total Cations + Cl^-

Symbols as in Figures 12 A and B


0300
0900
1500
2100
20 25 30 cyc
A
160
140
132
t Total Cations , mmol · Kg⁻¹
30 20 25 cyc
30 20 cyc 25
25 30 cyc 20
30 20 cyc 25
20
25
30
CYC
03 09 15 21
03 15 21 09
03 09 15 21
15 03 09 21
B
180
160
148
t Total Cations + Cl⁻, mmol · Kg⁻¹
20 25 30 cyc
30 20 cyc 25
20 25 30 cyc
30 20 cyc 25
20
25
30
CYC
03 09 15 21
03 15 21 09
03 09 15 21
15 03 09 21

stability with variations limited to one sampling period in most cases. Significant trends were observed in the following: (i) 20°C erythrocytic total cations + Cl^-; (ii) 25°C erythrocytic total cations; (iii) 25°C muscle total cations and total cations + Cl^-; and (iv) cycling temperature tissue total cations and total cations + Cl^-. In plasma and erythrocytes, cycling temperature values were more stable than those under constant temperature conditions.

(b) Comparisons made with respect to cycling versus constant temperature concentrations revealed the following patterns; (i) for both plasma parameters, the former was surpassed by the latter regardless of the static temperature values used; (ii) erythrocytic total cation levels of cycling fish were intermediate to those of statically-acclimated animals, in general and when corresponding static temperature values were used; (iii) total cations + Cl^- levels in the erythrocytes of cycling fish were equivalent to the lower static temperature readings but were surpassed by those static values paralleling the cycle; and (iv) although equivalent to the 20°C constant temperature group, cycling temperature muscle total cations and total cations + Cl^- were greater than those static temperature values at times and temperatures coinciding with the cycle itself.

c) Although irregularities were observed for each parameter, trends could be distinguished amongst the static temperature concentrations. In plasma, total cation levels decreased with increases in acclimation temperature, while the converse was true for total cations + Cl^-. Both erythrocytic parameters exhibited the following decreasing order in concentration: 30°C, 20°C and 25°C. The corresponding parameters in tissue decreased as follows: 25°C, 30°C and 20°C.

DISCUSSION

Discussion of the data gathered in this investigation will focus on two areas: modification in erythrocytic ion composition and possible influence(s) upon oxygen uptake and release and the general question of water-electrolyte balance. Under the first, temperature-induced alterations in hematological parameters and erythrocytic water and electrolyte content will be considered. Water-electrolyte balance will be dealt with in terms of plasma and muscle tissue changes with temperature.

Hematological Parameters

Representative hematological data from earlier studies has been tabulated in Table 3. Hemoglobin values ranged from a low of 4.6 ± 0.17 g$\cdot$100 ml^{-1} at 2°C to a high of 8.4 ± 0.04 g $\cdot$ 100 ml^{-1} at 35°C (Houston & Cyr, 1974). With a range of 6.54 ± 0.34 g $\cdot$ 100 ml^{-1} at 20°C to 8.81 ± 0.21 g$\cdot$100 ml^{-1} under the cycling thermal regime, the hemoglobin levels obtained in the present study correspond closely to those reported previously. In the instance of packed cell volume, values from this investigation (23.7 ± 1.1% at 20°C to 29.9 ± 1.0% at 20°C) were comparable to the low to mid-range literature values (total range being 21.1 ± 3.6 at 23°C (Houston & Rupert, 1976) to 44.7 ± 0.43 at 35°C (Houston & Cyr, 1974)).

The lack of consistent hematological response to temperature has been alluded to previously. This also characterized the goldfish used in this study. For example, static temperature hemoglobin and packed cell volume levels exhibited no consistent trend with acclimation temperature over the entire sampling period. The underlying factors which may account for these patterns of response have been discussed. However, it has also been pointed out that, at present, the factors regulating teleostean erythropoeisis, and the extent to which these are altered by environmental agents, are still largely unknown. Any attempt at an explanation at this time would, therefore, represent little more than speculation.

In the Review of Literature, reference was made to weight-specific differential hematological responses by the goldfish. Correlation analyses were therefore carried out for both packed cell volume and hemoglobin in relation to weight. Consistently significant correlations were encountered only between hemoglobin content and weight under the two lower constant thermal regimes (i.e., 20°C, 25°C). In general, specimen weight appeared to have little bearing on the hematological responses exhibited by these animals. Because of this, other data were not categorized by weight, or, in other words, source of origin.

Perhaps the most interesting aspect of this portion of the study was the difference in response seen in cycling temperature fish and those maintained under constant thermal conditions. In the case of both hemoglobin content and packed cell volume, the former displayed variations which were more diurnally stable and somewhat higher than those of the latter. Under conditions where oxygen demand and availability are changing, oxygen carrying capacity appears to be maintained at a relatively high constant level. This would suggest that these fish are responding to the high temperature portions of the cycle. While this mode of response is adaptively appropriate and would, in combination with increases in ventilatory and cardiac activity, amplify oxygen uptake, further potential advantage might be gained through modification of ionic modulators of hemoglobin-oxygen affinity and this is next considered.

Erythrocytic Water-Electrolyte Status

Discussion of acclimatory alterations in erythrocytic electrolyte concentrations should, however, be prefaced by some consideration of how such changes could be brought about. In addition, the manner in which such concentrations are expressed is of importance. These points will be dealt with in turn. Electrolyte concentration changes may be brought about in two ways; by modification in the partitioning of ions between the interior and exterior of the cell, and by changes in cell water content. Since erythrocytic water content exhibited very limited change with temperature, the changes seen in electrolyte levels are most reasonably attributed to alterations in the concentrations themselves. Electrolyte concentrations expressed as $mmol \cdot l^{-1}$, cell water are not accurate for all ions. In the instance of Na^+, K^+ and Cl^-, such value is acceptable. However, in the case of Mg^{2+} and Ca^{2+}, they are less realistic. This stems from the fact that the former group appears to occupy the total H_2O space of the cell (Gary-Bobo & Solomon, 1968), while of the latter, much of the Ca^{2+} and some of the Mg^{2+} is bound and therefore not dissolved in water (Bunn, et al., 1971). Consequently, the results will be discussed in terms of mmol electrolyte per litre of cells without reference to intracellular distribution.

A) Thermoacclimatory Responses in Erythrocytic Electrolyte Levels

With the exception of K^+, significant trends with increasing acclimation temperature could be distinguished in the constant temperature groups. Increases of 31.3-66% and 3.0-12.4% were found between 20°C and 30°C for N^+ and Cl^- respectively. Decreases in concentration between the same temperature range were noted in the case of Mg^{2+} and Ca^{2+}. The magnitudes of the latter were 10.2-17.9% and 65.5-75.7%

respectively. Although the differences observed were not always significant, the following order was obtained for K^+ levels : 20°C, 30°C and 25°C (highest to lowest).

Ion-hemoglobin ratios involving Na^+, Mg^{2+} and Ca^{2+} followed a pattern corresponding to their equivalent ion concentrations _per se_. K^+ : Hb and Cl^- : Hb ratios, did not vary significantly with temperature.

Changes in the Na^+ and Cl^- levels of statically-acclimated goldfish corresponded to those encountered by Catlett and Millich (1976), (see Table 5). The increase in Na^+ levels was, however, greater than that reported by Catlett and Millich. Variations in Cl^-, on the other hand, were similar. It should be noted that the temperature range employed by Catlett and Millich was 1°C to 21.5°C, whereas in this investigation it was 20°C to 30°C. Comparison of the values obtained by them at 21.5°C with those at 20°C in the present study indicate that Na^+ concentrations were somewhat higher and Cl^- substantially so. The rise in K^+ concentration (20%) over the temperature interval of 1°C to 21.1°C seen by Catlett and Millich, was not observed in this investigation. Potassium levels actually decreased between 20°C and 30°C, with the magnitude of this decrease being greatest between 20°C and 25°C (2.9 - 11.5%).

The changes in red cell ion content may have been of adaptive utility. Two general forms of adaptive response can be visualized in terms of ionic modulation of oxygen-hemoglobin affinity. The organism may alter composition so as to increase affinity and facilitate oxygen loading. This type of response appears most appropriate under circumstances in which temperature-related increases in oxygen demand are coupled with true hypoxia, i.e., reduction of ambient oxygen tension as well as content. Weber and Lykkeboe (1978) observed this in carp. Alternatively, under normoxic circumstances, which permit achievement of near-saturation hemoglobin oxygen-loading, reductions in affinity which improve oxygen release to tissues offer considerable advantage. Studies by Houston and Smeda (1978) on rainbow trout and carp suggest that this occurs at high temperatures in well-oxygenated waters. Environmental oxygen and temperature conditions applied in this investigation corresponded to the second set of circumstances referred to above, normoxic conditions coupled with increases in ambient water temperature.

In light of this, changes in erythrocytic electrolyte concentrations can be assessed in terms of their potential effects on hemoglobin-oxygen affinity. The most important ions in this sense are Mg^{2+} and Cl^- with K^+, Ca^{2+} and Na^+ being of lesser importance. Temperature-correlated changes for Mg^{2+} and Cl^- concentrations were inversely related; the former declining with increases in temperature, while the latter increased. These would be conducive to decreases in affinity. On the other hand, K^+ levels were higher at 20°C than at 25°C and 30°C. Although the differences seen were not always significant, these changes would be conducive to increases in hemoglobin-oxygen affinity; i.e., opposed to those which would be expected of Mg^{2+} and Cl^-. Na^+ and Ca^{2+} concentrations, however, were altered in such a manner as to strengthen the effects induced by Mg^{2+} and Cl^-. Therefore, the overall response indicated by these alterations in erythrocytic electrolyte levels would be a decrease in hemoglobin-oxygen affinity with temperature.

The interpretation of changes in red cell ion content appears straightforward. However, the situation becomes more complex when ion : hemoglobin ratios are considered. Alterations in Na^+ : Hb, Mg^{2+} : Hb and Ca^{2+} : Hb ratios tended to follow those of ion concentrations. Interestingly, K^+ : Hb ratios did not vary significantly with temperature and therefore mediate against the likelihood of affinity increases as suggested by K^+ concentrations. In the case of Cl^- : Hb, however, temperature-related variations were not significant; a contrast to changes in Cl^- concentrations. This suggests that Cl^- would not contribute to a decrease in oxygen affinity at higher temperatures.

It is of importance, however, to consider individual specimens as well as the trends expressed by the experimental groups. Figures 21A and B summarize values for 20°C animals and cycled animals at 20°C (03:00 hr) and 30°C fish together with cycled specimens sampled at 30°C, i.e., at 15:00 hr. In the former, red cell magnesium (mmol l^{-1}, cell H_2O) proved to be positively and significantly correlated with chloride; rbc $[Mg^{2+}]$ = 8.88 + 0.241 rbc $[Cl^-]$, (N = 70, r = 0.279, $P < 0.05$). In other words, increases in red cell chloride, which should reduce affinity, were linked to increases in magnesium content which would presumably oppose such effects. By contrast, at 30°C, an inverse relationship was apparent; rbc $[Mg^{2+}[$ + 10.89 - 0.011 rbc $[Cl^-]$. In short, at higher temperatures, variations in erythrocytic affinity would tend to facilitate oxygen release at the tissue level.

Also of interest in this regard is the relationship between Mg^{2+} : Hb and Cl^- : Hb ratios (see Figures 22A and B). At both 20°C and 30°C (constant plus cycled animals at appropriate temperature), direct and highly significant correlations were observed:

(1) 20°C (Mg^{2+} : Hb) = 0.601 + 0.072 (Cl^- : Hb), (N = 68, r = 0.479, $P < 0.01$)

(2) 30°C (Mg^{2+} : Hb) = 0.040 + 0.086 (Cl^- : Hb), (N = 84, r = 0.737, $P < 0.01$)

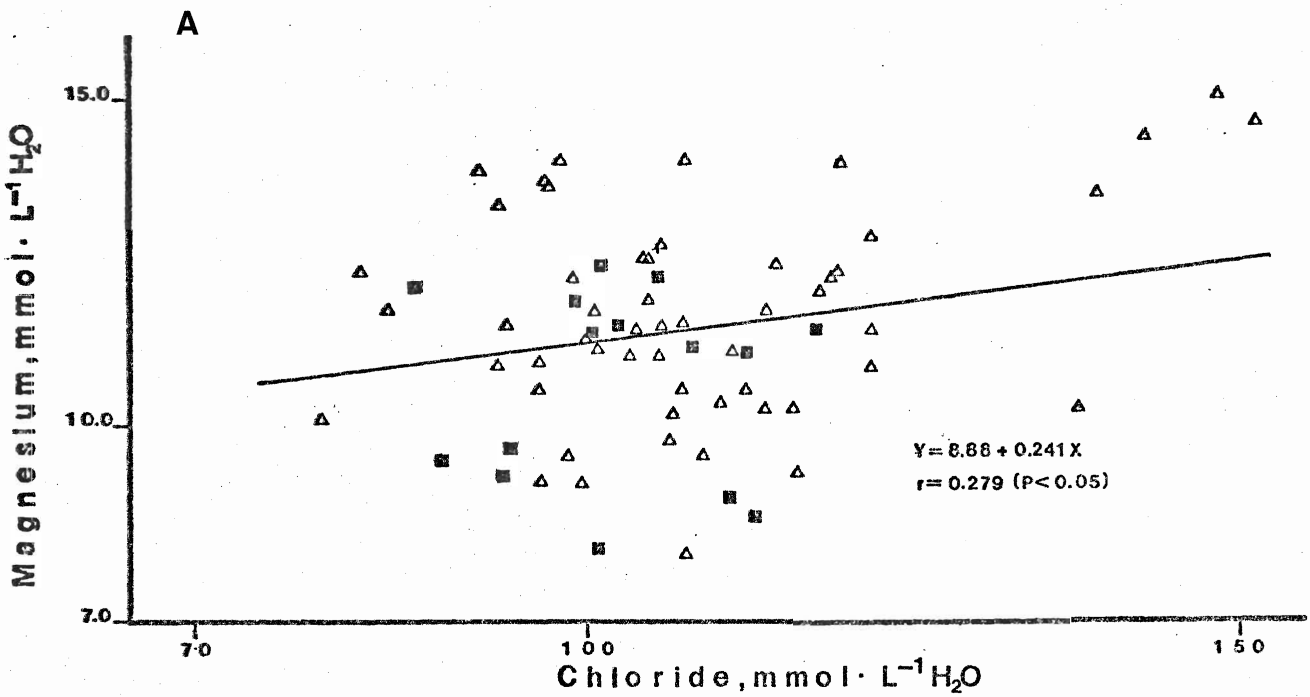
Here, increases in temperature are associated with substantial decreases in the value for Mg^{2+} : Hb associated with specific values for Cl^- : Hb; reductions in the order of 20 to 25% or

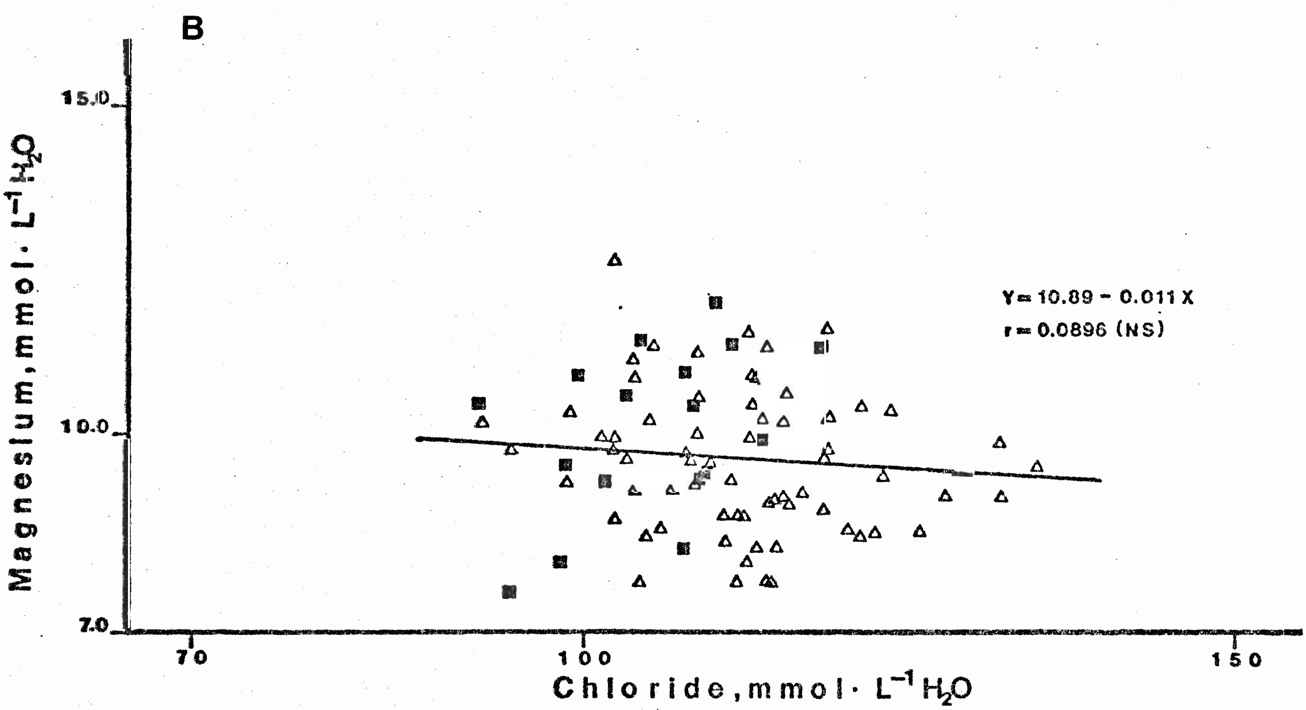
Figures 21 : Relationship between erythrocytic magnesium and chloride ($mmol \cdot l^{-1}$, cell H_2O) in the goldfish.
A & B

A Data are for 20°C constant temperature readings (open triangles) and cycling temperature values obtained at 03:00 hr (20°C), (closed squares).

B Data are for 30°C constant temperature readings (open triangles) and cycling temperature values obtained at 15:00 hr (30°C), (closed squares).

In both figures, the best fitting lines, their equations and the significance level of the correlation are included.


A
15.0
10.0
7.0
70
100
150
Magnesium, mmol · L⁻¹ H₂O
Chloride, mmol · L⁻¹ H₂O
Y = 8.88 + 0.241 X
r = 0.279 (P < 0.05)

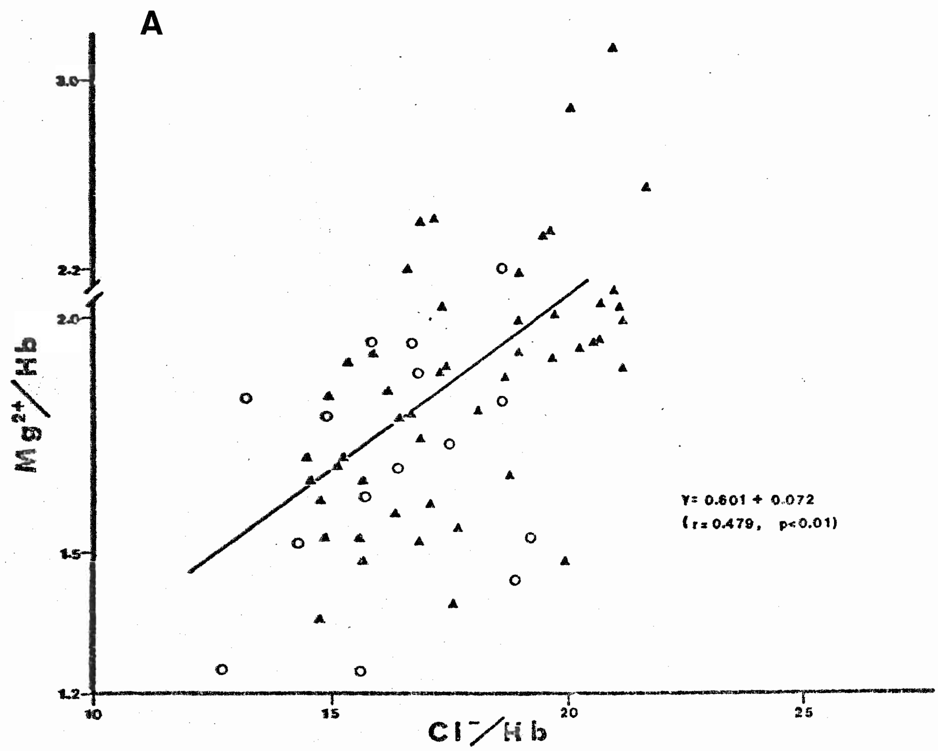

B
15.0
10.0
7.0
70
100
150
Magnesium, mmol · L⁻¹ H₂O
Chloride, mmol · L⁻¹ H₂O
Y = 10.89 − 0.011 X
r = 0.0896 (NS)

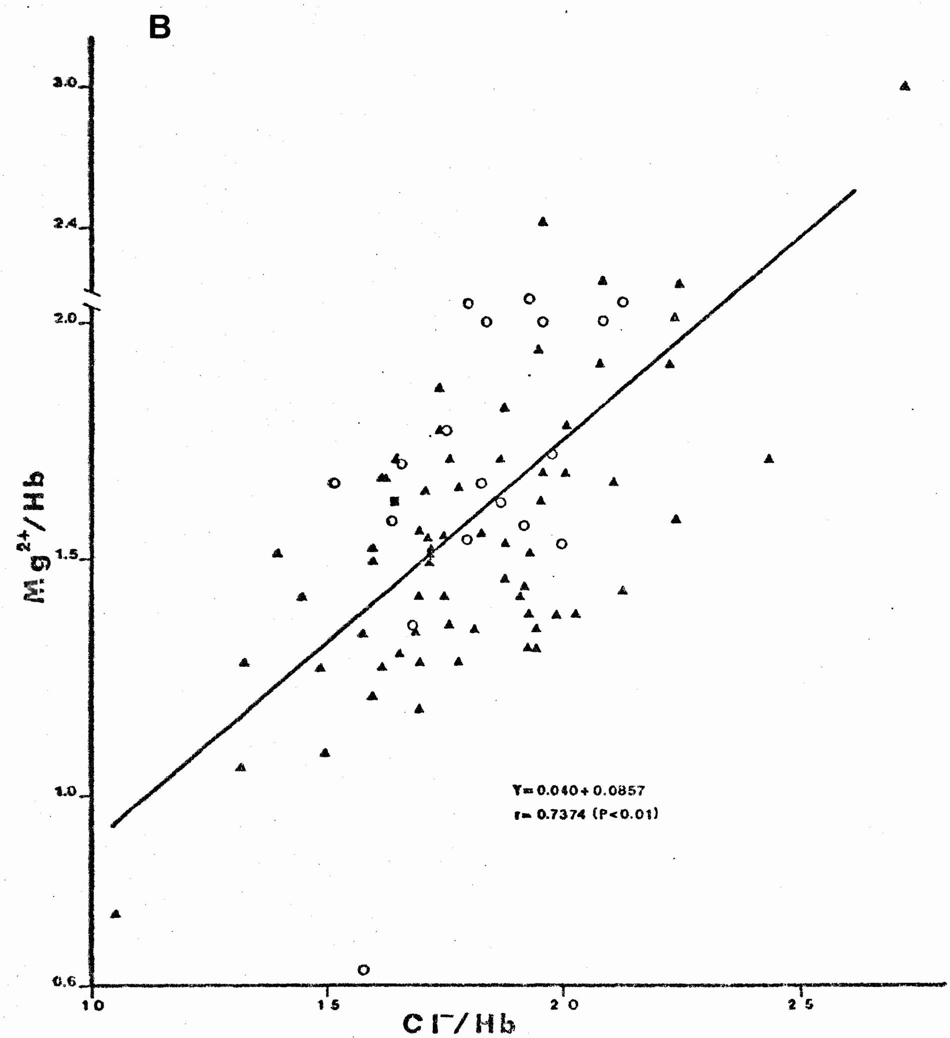
Figures 22 : Relationship between erythrocytic Mg^{2+}:
A & B Hb and Cl^-:Hb ratios ($mmol \cdot l^{-1}$, packed cells/$mmol \cdot l^{-1}$, packed cells).

A Data are for 20°C constant temperature readings (closed triangles) and cycling temperature values obtained at 03:00 hr (20°C), (open circles).

B Data are for 30°C constant temperature readings (closed triangles) and cycling temperature values obtained at 15:00 hr (30°C), (open circles).

In both figures, the best fitting lines, their equations and the significance of the correlation are included.


A
3.0
2.2
2.0
1.5
1.2
10
15
20
25
Mg2+/Hb
Cl⁻/Hb
Y= 0.601 + 0.072
(r=0.479, p<0.01)


B
3.0
2.4
2.0
1.5
1.0
0.6
10
15
20
25
Mg2+/Hb
Cl⁻/Hb
Y=0.040+0.0857
r= 0.7374 (P<0.01)

more. Again, this suggests that alterations in red cell chloride and magnesium would tend to favour reductions in hemoglobin-oxygen affinity at higher temperatures.

Thus, the changes in erythrocytic electrolyte content exhibited by goldfish tend to resemble those reported by Houston and Smeda (1979) for the carp. In both cases, these would tend to prompt reductions in hemoglobin-oxygen affinity at higher temperatures, facilitating the release of oxygen at the tissue level, and compensating for increased oxygen demand.

B) Water-Electrolyte Status Under the Cycling Temperature Regime

Red cell Na^+, K^+ and Ca^{2+} concentrations of cycling animals exhibited distinct variations with time and cycle temperatures. The following trends could be distinguished :

1) K^+ levels tended to decrease as temperatures became higher;
2) Na^+ concentrations at 03:00 hr increased thereafter and stabilized;
3) a low Ca^{2+} level ($P < 0.05$) occurred at 15:00 hr;
4) water content increased with temperature.

Variations in ion : hemoglobin ratios were more prevalent and apparent than were changes in ion concentrations. Variations in Mg^{2+}:Hb and Ca^{2+}:Hb ratios resembled those of

Mg^{2+} and Ca^{2+} concentrations. Na^+ : Hb increased with increasing temperature, with a notably high value at 15:00 hr. Unlike Cl^- concentrations, Cl^- : Hb ratios exhibited a high at 15:00 hr while K^+ : Hb ratios peaked at 03:00 hr and 15:00 hr, i.e., at the low and high temperatures.

In terms of hemoglobin-oxygen affinity modification, several features are of interest. Changes in erythrocytic electrolyte concentrations are such that modifications were not of great magnitude, suggesting that modifications in hemoglobin-oxygen affinity with increasing temperature were limited. Of the functionally-more important ions, only K^+ underwent marked changes and these were as to increase affinity. On the other hand, the variation in Na^+ and Ca^{2+} would tend to produce the opposite effect. The influences of Na^+ and Ca^{2+} upon affinity are not of major importance. If these changes are related to hemoglobin levels, however, a somewhat different picture emerges,for variations in all ion:hemoglobin ratios point to decreased affinity at the highest cycle temperatures. Thus, if the overall trends are examined, the data suggest that hemoglobin-oxygen affinity is modified in a manner conducive to the release of oxygen to tissues at times when their oxygen demands are elevated. This form of response corresponds to that observed in the carp (Houston & Smeda, 1979).

C) Diurnal Variations in Water-Electrolyte Status Under Static Versus Cycling Temperature Conditions

Two general features characterized erythrocytic electrolyte levels of statically-acclimated fish. Concentrations at such constant temperature were either relatively stable, or, if variations did occur, they were not consistent in the sense of time of occurrence of maxima and minima. On the other hand, in cycled animals, such electrolyte alterations as occurred were usually correlated with cycle temperature. Thus, these animals tended to exhibit more temporally-consistent patterns of compositional change than were apparent in the constant temperature groups. This tended to be the case with ion : hemoglobin ratios as well.

Observations of this kind lead one to consider whether eurythermal animals are, in fact, pre-adapted to temperature cycles and, as a corollary, whether deviations encountered under constant conditions represent artifacts, i.e., that the red cell behaves as it would under a cycling condition even when the animals are exposed to constant thermal regimes. The implication is clearly that red cell transport mechanisms are intrinsically programmed to respond to cyclic thermal regimes whether such conditions exist or are absent. Presumably, the selective forces acting in this instance involves maintenance of an appopriate hemoglobin micro-environment in relation to oxygen demand. Continuance of transport activity in the absence of external thermal stimuli could well account for the inconsistent diurnal pattern exhibited by animals acclimated to constant temperatures.

It would be of great interest to examine this in a more steno-thermal species such as salmonids which normally encounter less variation. It would, of course, be of considerable interest to examine the consequences of exposure to fluctuations of different amplitudes around baselines other than that employed.

D) <u>Total Cations and Total Cations + Cl^- in Cycling and Statically-Acclimated Goldfish</u>

Except for the minor, but significant difference reported for total cation concentrations, both total cation and total cation + Cl^- were stable under cycling temperature conditions. This suggests that, although individual electrolyte concentrations may vary with temperature, the osmolality of the intra-erythrocytic environment is maintained reasonably constant. Presumably, this can be accounted for in terms of temperature-induced alterations in red cell transport enzyme activities. By contrast, under static temperature conditions these values exhibited greater variability. In general, the trends seen in this case formed no consistent pattern. Within the context of cycling versus static temperature alterations, the above observations suggest that pre-adaptation to cycling temperatures is characteristic of these animals. This is reflected in the constancy of the overall erythrocytic ionic environment in the former, but not in the latter. Further discussion on this topic is not warranted since it has already been covered previously,

In comparing cycling temperature values to those of statically-acclimated fish, the following trends were observed : 1) total cation concentrations of cycled temperature specimens were found to be intermediate with respect to static temperature values in general, or those paralleling the cycle itself and 2) total cations + Cl^- values were, in general, below those of constant temperatures corresponding to the cycle while, in the case of a more generalized comparison, they were equivalent to the lower static temperature readings recorded. This leads to the suggestion that, although individual cation species in cycling temperature animals may be altered with respect to those seen under constant thermal conditions, the overall pattern is indicative of reciprocal changes which tend to affect one another.

Trends among the static temperatures alone were similar for both total cations and total cations + Cl^-. The patterns observed were characterized by levels at 25°C that were generally lower than under the other two thermal conditions. However, it should be noted that, in many cases, significant differences were not encountered. In addition, the trends observed did not coincide with any of those shown by single ion species, other than K^+. This suggests that the individual alterations exhibited by erythrocytic electrolytes interact in a complex manner to produce the patterns observed here.

Mean concentration values were used to estimate the magnitude of changes in anion deficit with temperature, a parameter which is suggestive of HCO_3^- concentration (total cations - Cl^- (see Table 8). No consistent diurnal or thermal trends were noted amongst the static temperatures. However, cycling temperature values exhibited a drop in level corresponding to the high temperature of the cycle. Given that CO_2 in all groups is constant, this suggests that, as the cycle temperature rises from 25°C to 30°C, the red cell becomes relatively more acidic, implying that Bohr effects may be enhanced and, therefore, that oxygen unloading is augmented.

E) Water-Electrolyte Variations Associated with Diurnal Temperature Cycles

Exposure of goldfish to diurnal temperature cycles resulted in alterations in erythrocytic electrolyte content which were, in some respects, distinct from those seen under static conditions. This was most pronounced when comparisons were made with static temperature values paralleling the cycle. Water content, on the other hand, was not significantly affected by exposure to cycling temperatures.

Consistent differences between static and cycling temperature values were limited to Mg^{2+}, Ca^{2+} and Cl^- concentrations. Under cycling temperature conditions, Mg^{2+}

TABLE 8 : Mean erythrocytic anion deficit levels (mmol l^{-1}, cell H_2O) in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods.
Values obtained from the equation (total cation ($mmol \cdot l^{-1}$, cell H_2O) - Cl^- ($mmol \cdot l^{-1}$, cell H_2O)).

ERYTHROCYTIC ANION DEFICIT ($mmol \cdot l^{-1}$, cell H_2O)

TIME (hr)	TEMPERATURE			
	20°C	25°C	30°C	Cycle (25 ± 5°C)
03:00	77.32	69.37	74.12	71.61
09:00	74.70	55.13	67.42	70.69
15:00	68.27	51.06	61.28	65.50
21:00	60.74	54.09	62.82	70.97

levels were intermediate to those of the constant thermal groups; Ca^{2+} levels were equivalent to those of the 30 C group; Cl^- concentrations were somewhat below those of statically-acclimated animals. The above relationships were also seen in the equivalent ion : hemoglobin ratios.

Utilization of constant temperature concentrations corresponding to those of the temperature cycle revealed distinguishable patterns. In the case of K^+ and Mg^{2+} levels, cycling temperature animals exhibited values exceeding those under static thermal conditions. The extent of significant differences was high in the former case and somewhat less in the instance of Mg^{2+}. By contrast, Na^+, Ca^{2+} and Cl^- concentrations exhibited a converse trend and all three were significant. Only in the case of Ca^{2+} and Na^+ did ion : hemoglobin ratios follow the pattern exhibited by ion concentrations. K^+ : Hb and Cl^- : Hb appeared to mimic patterns displayed by ion concentrations. However, these were not consistent in terms of the overall trend exhibited and the significant differences encountered. Mg^{2+} : Hb ratios in cycling temperature fish were intermediate and not significantly different from their static temperature counterparts.

The most appropriate means of assessing difference between constant temperature and cycled animals involves comparisons equivalent in time as well as temperature. When this was done an interesting situation was observed. Upon exposure to cycling temperatures, Cl^- and Mg^{2+} concentrations varied in a fashion which would be conducive to enhanced affinity, while K^+ levels suggested a decrease in affinity. However, the differences in K^+ concentrations were not significant. Na^+ levels suggested that affinity would be higher in cycling as compared to statically-acclimated animals. Differences in Ca^{2+} levels were suggestive of a decrease in affinity. Although differences in Na^+ and Ca^{2+} were significant, their influence on oxygen-hemoglobin affinity is, as previously noted, not pronounced. This data, therefore, suggest the likelihood of increases in hemoglobin-oxygen affinity with acclimation to diurnal temperature cycles.

Consideration of ion : hemoglobin ratios, however, leads to a somewhat different conclusion. No significant differences were observed in Mg^{2+} : Hb, Cl^- : Hb and K^+ : Hb and this suggests that hemoglobin-oxygen affinity is not altered by cycling temperatures. The opposed trends in Na^+ : Hb and Ca^{2+} : Hb ratios lends support to this as well.

In summary, the differences between cycling and constant temperature animals in specific erythrocytic electrolyte levels suggest the likelihood that an increase in hemoglobin-oxygen affinity would be brought about by exposure to diurnal temperature cycles. However, when these responses were considered in relation to hemoglobin changes, no obvious relationship was apparent. Therefore, although hemoglobin-oxygen affinity may be altered, the functional significance of this is not of overriding importance.

Water-Electrolyte Balance in the Goldfish

A) Plasma

The most notable feature encountered in this phase of the study was the diurnal stability exhibited under cycling, as compared to constant thermal conditions. Only in the instance of Na^+ and Ca^{2+} were significant variations detected in the cycling group. However, the increase in Na^+ (21:00 hr) was no greater than the alterations observed under the static temperatures. This change amounted to only an $\sim$3.0% increase in concentration. On the other hand, the rise in Ca^{2+} concentration ($\sim$ 13%) was both highly significant and also corresponded to the high temperature of the cycle. Diurnal variability shown by statically-acclimated animals was most notable for the inconsistent patterns observed.

As pointed out earlier, results of this kind are consistent with the postulation that these animals may be pre-adapted to cycling thermal conditions. This is evidenced by the stability of both electrolyte and water content in cycling as compared to constant temperature animals.

Previous studies have rarely considered diurnal variation in water-electrolyte balance and their basis has consequently not yet been examined. However, the findings of Henry (1980), although concerned with the stenothermal rainbow trout, are pertinent. Trout respond to the rising phase of the temperature cycle by increasing ventilatory flow and rate and cardiac rates. Indirect evidence of increased cardiac output was also reported. These systemic alterations should augment both water influx and ion efflux and should prompt water loading and electrolyte loss at higher temperatures. Nevertheless, both remain stable in the case of the goldfish. Clearly, some form of compensatory response must occur. At the kidney level, urinary flow rates can be increased to alleviate water loading, but at the same time, result in elevated renal electrolyte losses. To counteract branchial and renal ion losses, alterations in the gill level may be involved. The two most abundant mechanisms involve: 1) branchial ionic permeability adjustments limiting electrolyte efflux; and 2) increased active recruitment of ions from the environment.

Although they involved non-diurnal acclimation studies or were the consequences of abrupt temperature changes, the following investigations are pertinent to the findings of this study. At 5°C, goldfish replace their body water at a rate of 25.2% hr^{-1} (Isaia, 1972). With acclimation to 25°C water turnover increased to 135% hr^{-1}; a more than five-fold increase. Transfer of 16°C acclimated goldfish to

$6^{\circ}C$ resulted in a decrease of approximately 60% in sodium uptake, presumably to counteract passive losses of this ion (Maetz, 1972). Sodium and chloride uptake rates of Arctic grayling Thymullus arcticus, rose by 20% and 258% respectively following transfer from 10° to $17^{\circ}C$ (Cameron, 1976).

In an attempt to account for increases in uptake rates at higher temperatures, McCarty and Houston (1977) and Houston and McCarty (1978) proposed a modified form of the "chloride" cell model of Maetz (1971). The various exchange and transport components of this were outlined in detail in the Review of Literature. The basic proposition involved enhancement of branchial and renal (Na^+ / K^+)-ATPase activities to increase Na^+ uptake and erythrocytic carbonic anhydrase to elevate Cl^- absorption; these being superimposed on base levels of Na^+/H^+, NH_4^+ and Cl^-/HCO_3^- exchange uptake and recovery mediated by thermostable branchial and renal carbonic anhydrase systems.

Examination of temperature-related plasma electrolyte alterations in the statically-acclimated animals of this study revealed some interesting features. Consistent trends were encountered for Na^+, Mg^{2+} and Cl^- only. Na^+ and Cl^- tended to be inversely related; the former decreasing, while the latter increased with rising temperature. These changes tended to be larger for Cl^- than for Na^+. Mg^{2+} levels in $25^{\circ}C$ goldfish were significantly higher than those of animals held at 20°C and 30°C.

Previously reported values for plasma electrolyte concentrations in goldfish have been compiled in Table 9. Only the major ions, Na^+ and Cl^- and K^+ have received attention in this respect. Values obtained in the present investigation correspond to those of the literature in the following respects : (1) Na^+ and K^+ concentrations were equivalent to the mid- to lower range and (2) Cl^- levels were found in the mid- to upper readings. Over equivalent thermal ranges, Na^+ concentrations changed in the fashion reported by Murphy and Houston (1974), decreasing with increases in acclimation temperature. The increase seen in Cl^- levels noted in this study, corresponded to that noted by Mackay (1974) for goldfish maintained under equivalent temperature conditions in demineralized water. Plasma K^+ concentrations did not vary in the manner observed in earlier studies. No consistent trends were observed, whereas increases in K^+ concentration with temperature were recorded earlier.

Earlier investigations, using goldfish, did not involve determinations of plasma Mg^{2+} and Ca^{2+}, and therefore, comparisons of present values can be made only with those obtained for other eurythermal species. In fact, few values for plasma Mg^{2+} and Ca^{2+} have been reported. The most complete are those of Houston and Smeda (1979) and Houston, _et al_., (1970) for carp and Grigg (1969) for the brown bullhead,

TABLE 9 : Plasma Na^+, Cl^- and K^+ : Representative Values in Thermally-Acclimated Goldfish

TEMPERATURE (°C)		Na^+ (mmol/l)	Cl^- (mmol/l)	K^+ (mmol/l)	Reference
30		155±8	78.5±1.5	8.3±0.3	Heinicke & Houston, 1965b.
20		169±9	82.0±0.8	6.1±0.6	
25		165.7±1.9	112.1±1.4	3.26±0.12	Prosser, *et al.*, 1970
15		162.5±2.0	121.5±1.2	3.10±0.13	
5		154.7±2.1	117.2±1.8	2.38±0.05	
20		147.3±3.0	123.5±7.5	5.3±0.5	Umminger, 1971
10		137.0±1.3	115.6±1.9	4.2±0.6	
0.5		130.4±2.2	104.5±2.0	3.0±0.1	
24	tap water	143±2.2	113 ±0.9	4.2±0.3	MacKay, 1974
14		147±1.3	117 ±1.1	3.1±0.1	
6.5		125±4.7	90±7.4	3.3±0.1	
30	tap water	130±1.3	100±1.2	3.3±0.2	
20		130±1.4	102±1.1	2.3±0.1	
10		134±1.3	102±1.0	2.2±0.2	
30	demineralized water	115±1.5	95±2.3	2.4±0.2	
20		104±4.6	76±6.3	2.0±0.2	
10		92±2.2	66±2.1	2.7±0.2	
35		123.2±1.6	102.6±1.8		Murphy & Houston, 1974
25		131.1±1.8	116.7±2.4		
15		139.1±1.3	117.9±2.8		
5		122.8±4.1	104.3±2.4		

Ictalurus nebulosus. These are summarized in Table 10. Mg^{2+} levels in the present study were somewhat below those of the carp (Houston, *et al*., 1970), while Ca^{2+} concentrations were equivalent to those of animals held in a similar thermal range. In addition, the following features emerged : (1) Mg^{2+} levels in the carp remained stable or were elevated at 2°C, whereas goldfish exhibited maximum values at 30°C and (2) the inconsistent variations in goldfish Ca^{2+} were unlike the increases, or stability exhibited by other species.

Water content exhibited no consistent variation in relation to acclimation temperature. Catlett and Millich (1976) reported that plasma water levels in goldfish increased with reductions in temperature. However, this was encountered only at the lowest temperatures (5°C and 1°C) employed and these were well below those of this study. Furthermore, Houston and Smeda (1979) also found no significant change in plasma water content in carp over a temperature range of 2°C to 30°C.

The variability in water electrolyte status seen in the goldfish of this study, and notably the fact that, under stable temperature conditions ion and water levels do not generally exhibit diurnally-consistent patterns of change, lends indirect support to the suggestion that these fishes may be incapable of maintaining a steady-state condition under such circumstances. As noted earlier, they may be genetically

TABLE 10 : Plasma Mg^{2+} and Ca^{2+} : Representative Values in Thermally-Acclimated Carp and Brown Bullhead

SPECIES	TEMPERATURE (°C)		Mg^{2+}	Ca^{2+}	UNITS	REFERENCE
Carp (*Cyprinus carpio*)	7	(S)*	0.83±0.02	1.42±0.12	mmol/1	Houston, et al., 1970
	2	(S)	1.15±0.06	0.98±0.10		
	27	(W)	0.88±0.04	1.91±0.11		
	7	(W)	-	-		
	33	(F)	1.13±0.03	2.54±0.09		
	27	(F)	1.21±0.05	2.44±0.10		
	17	(F)	1.23±0.03	2.12±0.10		
	4	(F)	1.19±0.02	1.94±0.04		
Carp (*Cyprinus carpio*)	30		2.47±0.12	4.22±0.13	mEq/1	Houston & Smeda, 1979
	16		2.29±0.08	4.30±0.13		
	2		2.86±0.07	3.70±0.12		
Brown bullhead (*Ictalurus nebulosus*)	24		-	2.23	mEq/1	Grigg, 1969
	9		-	2.71		

* S = Summer; F = Fall; W = Winter

programmed for regulation under circumstances in which diurnal temperatures vary in a regular pattern. For an organism which has evolved under such circumstances, constant diurnal temperatures, at least during the summer months, may represent very unnatural conditions.

Comparisons made between static temperature values and those of fish held under a cycling thermal regime revealed that, for Na^+ and Cl^-, the former exceeded the latter. This became even more apparent when static temperature readings corresponding to the temperature cycle were utilized. Mg^{2+} concentrations were found to be intermediate with respect to those of statically-acclimated animals, while Ca^{2+} and K^+ were not significantly different. Thus, plasma water-electrolyte changes associated with exposure to the temperature cycle were such that overall stabilization took place at levels equivalent to, or below those of the corresponding static temperature groups.

The relationship of the present findings to those of Toews and Hickman (1969) for rainbow trout warrants comment at this time. As noted previously, the latter study was characterized by a number of procedural inadequacies. However, their data are unique from the standpoint that they represent the consequences of the only other study employing cycling as well as static temperature conditions. With constant temperature conditions, the following trends with rising temperature were obtained: (1) water content levels remained stable; (2) plasma Na^+ and Cl^- increased 32.2% and 38.4% respectively; the former over the $8^\circ C - 18^\circ C$ temperature range, the latter from $8^\circ C$ to $12^\circ C$; and (3) K^+ exhibited a 37% decrease between 8° and $18^\circ C$. Cl^- levels in goldfish displayed the same pattern, but of lesser magnitude ($\sim$4.0%). Other parameters did not behave as reported by Toews and Hickman. K^+ and water content did not vary consistently with temperature, and Na^+ levels actually decreased with increases in temperature. These differences may have resulted from the sampling method used by Toews and Hickman for constant temperature groups. Sampling was conducted at the same times as that of cycled temperature specimens.

Under cycling temperatures, the following relationships were noted between the two studies: (1) water content and K^+ concentrations were stable in both; (2) trout Na^+ levels displayed a 23.8% decrease between 10° and $18^\circ C$ on the rising phase of the cycle, while goldfish concentrations were stable except for the rise (2.9%) at 21:00 hr; and (3) goldfish Cl^- values were stable, while that of the rainbow trout peaked at $14^\circ C$ on the rising phase of the cycle and then decreased on the falling phase. The greater variability and the inconsistent trends in results for trout may be related to the cycle used, as this included a spiking increase rather than sine wave. A thermal regime of this kind entails much higher rates of temperature change and the fishes may be unable to compensate. The authors believed that a steady-state condition was never attained, and represented a physiological compromise to the situation. They concluded that to demonstrate this point conclusively, sine-wave temperature cycles should be utilized. The water-electrolyte stability of goldfish under such circumstances can be taken to indicate that a steady state is, in fact, achieved.

An interesting feature of the data in both studies emerged when cycling versus static temperature values were examined in relation to plasma electrolyte levels. Toews and Hickman (1969) found that Na^+, K^+ and Cl^- concentrations in cycling trout were similar to those of fish maintained at 8°C and 10°C. This was not a surprise, considering that the fishes spent most of their time at the lower temperatures of the cycle. In the present situation, no such pattern was noticeable and cycled temperature values were found, in general, to be similar to, or below those of statically-acclimated fish. It would be of interest to examine this further using cycles of different amplitudes around different baselines.

Taking the sum of cations + Cl^- as suggestive of osmolality, this permits comparison with osmolality values for goldfish in the literature under a variety of experimental circumstances (see Table 11). Two exactly opposite patterns with few intermediates can be distinguished from the latter values; (1) an increase with rising temperature (Mackay, 1974, demineralized water; Catlett & Millich, 1976); or (2) decreases under such conditions (Umminger, 1971; Mackay, 1974, tap water). The situation in statically-acclimated fish of this study was one of apparent stability with few differences encountered with respect to temperature. Furthermore, no obvious or consistent diurnal patterns and relatively little variation was seen over the sampling period. The actual 'osmolality' values obtained were intermediate to those reported by Mackay (1974) for goldfish held in demineralized water and all others reported. In cycled animals, "osmolality" was significantly below that seen under static conditions. They also exhibited minor and inconsistent diurnal fluctuations with values corresponding to the high temperature readings for goldfish maintained in demineralized water (Mackay, 1974). The apparent general effect of cycling temperatures, however, was an overall decrease in plasma osmolality.

Total cation concentrations in the plasma of these goldfish displayed the same features as those for total cations + Cl^-. Under static temperature conditions, variation with temperature did not follow any distinguishable pattern in that diurnal fluctuations were both minor and inconsistent.

TABLE 11 :Plasma Osmolality : Representative Values and Methods of Determinations in Thermally-Acclimated Goldfish.

TEMPERATURE (°C)	PLASMA OSMOLALITY (mOsm/l)	METHOD OF DETERMINATION	REFERENCE
20	309 ± 4	Melting point*	Umminger, 1971
10	299 ± 6		
0.5	341 ± 6		
30 tap water	265 ± 2	Freezing point depression	Mackay, 1974
20 tap water	270 ± 3		
10 tap water	271 ± 4		
30 demineralized water	246		
20 demineralized water	226		
10 demineralized water	204		
21.5	294.60±2.06	Melting point**	Catlett & Millich, 1976
10	288.40±2.32		
5	275.60±4.06		
1	270.00±2.73		

* utilized a Clifton Technical Physics Biological Cryostat/ Nanolitre Osmometer.

** compared to reference standards; melting time plotted against osmolality.

Houston and Smeda (1979) reported a small (5.6%) but significant decrease in the plasma total cation content of carp over the temperature range of $2^{\circ}C$ to $30^{\circ}C$. The diurnally-stable cycling temperature sums of cations were below those of the constant temperature groups. This feature was also apparent when static temperature values corresponding to the cycle were utilized. Exposure to a cycling thermal regime resulted in lower total plasma cation concentrations, but this difference was not as pronounced as in the case of total cations + Cl^-.

B) Muscle

The stability seen in the plasma water and electrolyte levels of cycling as compared to statically-acclimated animals was not as obvious in muscle tissue. Minor, but frequently significant diurnal variations were encountered for Na^+, Mg^{2+}, Ca^{2+}, Cl^- and water content values. In most instances, however, only one sample group was involved. Except for Ca^{2+} and water content, the trends encountered did not show any significant patterns with respect to the temperature cycle. The two aforementioned parameters, however, displayed peak values corresponding to the high temperatures of the cycle. K^+ levels exhibited the same general pattern, but the fluctuations were much greater. Total cations and total cations + Cl^- were also notable for peak values at the high temperatures of the cycle.

These features of muscle water-electrolyte balance coupled with the corresponding patterns seen in plasma, suggest a relatively tighter control of plasma composition. It is quite possible that the stable plasma water-electrolyte status encountered under cycling temperatures would provide an environment conducive to optimal erythrocyte functioning. However, an appropriate cellular environment must be equally important. From the standpoint of cellular metabolism, inorganic ions are known to be effective modulators of enzyme activity (Bygrave, 1967). Hochochka and Somero (1973) have described a variety of temperature-induced responses at the enzyme level in a number of teleosts, including the goldfish. In such cases, ions were shown to be important in the role of enzyme activity modulation.

It will be obvious that alterations in muscle concentrations do not provide an adequate description of changes in cell ion content with temperature. Three possibilities can, however, be considered. Tissue concentrations do not take into account possible variations in extracellular phase volume (ECPV). It is possible that increases in tissue ion concentrations of predominantly extra-cellular ions such as Na^+ and Cl^- reflect increases in ECPV and not increases in cellular concentration. Cellular ion concentrations may increase but, in the case of ions such as

Mg^{2+} and Ca^{2+}, may lead to no effective changes due to binding. Finally, increased cellular ion concentrations may result in the elevation of effective concentrations and be adaptively important in the modulation of enzyme activity. The evidence thus far obtained does not permit a conclusion with respect to any of these possibilities. Nevertheless, it does not necessarily follow that overall muscle electrolyte concentrations are indicative of a situation in which ion balance is not well regulated. A prior study (Mearow, 1979) on rainbow trout has, in fact, shown that in this stenothermal species both ECPV and cellular ion concentrations are modified with temperature. Mearow suggested that water and electrolyte levels may have been selectively altered to provide optimum circumstances for cellular function at specific temperatures.

In statically-acclimated animals of this study, the most consistent feature encountered was the maximum concentrations seen at 25°C. Na^+, K^+, Mg^{2+} and Ca^{2+} were all characterized by this, as were total cations and total cations + Cl^-. Cl^- concentrations tended to be higher at 20°C than they were under the two other static conditions, while maximum water content appeared to be associated with the 30°C thermal regime.

Few studies have been conducted on muscle water-electrolyte status in relation to temperature (see Table 12). Na^+ levels

Muscle Tissue

TABLE 12 Water and Electrolytes : Representative Values in Thermally-Acclimated Goldfish and Carp

SPECIES	TEMPERATURE (°C)	Na^+ (mmol/kg)	K^+ (mmol/kg)	Cl^- (mmol/kg)	Mg^{2+} (mmol/kg)	Ca^{2+} (mmol/kg)	WATER CONTENT (g/kg)	REFERENCE
Goldfish	25	47.6 ± 2.8	102.7 ± 3.7				818±5.4	Prosser,
Carassius	15	50.5 ± 4.9	100.2 ± 2.9				812±4.0	*et al.*,
auratus	5	44.0 ± 2.8	97.8 ± 4.0				819±3.0	1970
Goldfish	30	4.7 ± 0.8	115 ± 6	17.4±0.9			808±4	Heinicke
Carassius	20	7.7 ± 0.5	104 ± 4	19.3±1.4			807±2	& Houston
auratus								1965b
Goldfish	25						798±7.1	Das, 1967
Carassius	5						774±6.8	
auratus								
Carp	7 (S)*	12.4 ± 1.1	20.0 ± 2.0	10.0±0.7	2.3 ± 0.4	14.5±3.1	828.8 ±6.4	Houston,
Cyprinus	2 (S)	7.7 ± 0.5	28.1 ± 1.6	7.3±0.4	4.2 ± 0.2	14.7±2.6	813.3 ±2.9	*et al.*,
carpio	27 (W)	9.2 ± 1.2	32.1 ± 1.9	7.5±0.5	5.3 ± 0.3	12.3±2.3	776.1 ±6.5	1970
	7 (W)	8.3 ± 0.5	38.2 ± 2.0	6.8±0.3	4.7 ± 0.4	13.0±1.6	776.3 ±1.7	
	33 (F)	-	45.1 ± 2.4	8.6±0.2	5.1 ± 0.4	14.7±1.7	792.4 ±1.4	
	27 (F)	14.0 ± 1.6	-	8.0±0.5	4.8 ± 0.2	11.3±0.9	792.1 ±1.5	
	17 (F)	16.1 ± 0.8	65.1 ± 3.4	6.9±0.2	4.7 ± 0.1	10.4±1.0	797.2 ±1.5	
	4 (F)	-	-	8.0±0.2	-	-	804.4 ±1.3	

* S = Summer; F = Fall; W = Winter

reported here fall between those reported by Prosser, et al., (1970) and Heinicke & Houston (1965b). K^+ concentrations correspond well with those in the literature, while Cl^- values were somewhat below those of Heinicke and Houston (1965b). Water content levels were similar to those of Das (1967), but were below the values of Prosser, et al. (1970) and Heinicke and Houston (1965b). Comparable values for Mg^{2+} and Ca^{2+} in the goldfish have not been reported. However, values are available for the eurythermal carp (Houston, et al., 1970). In the former case, present values were higher than those for carp, while in the case of Ca^{2+}, the levels were found to be below or equal to the lower concentrations encountered (17°C and 27°C; fall animals).

The information in Table 12 permits for some comparison of apparent temperature-related changes in composition. However, only Heinicke and Houston (1965b) and Houston, et al. (1970; fall series) used thermal ranges equivalent to that of this study. Prosser, et al. (1970) reported a mid-point temperature (15°C) peak for Na^+ similar to that found herein. Over the equivalent temperature range, Heinicke and Houston (1965b) and Houston, et al. (1970) found a decrease in this ion species with temperature. In the goldfish, previous studies found K^+ to increase with temperature while in the carp, the opposite trend was shown. In the present study, K^+ concentrations did not follow either pattern. Heinicke and

Houston (1965b) (goldfish) observed a decrease in Cl^- with temperature, while Houston, _et al_. (1970) (carp) reported a low value at 17 °C. These correspond to the lower Cl^- concentrations encountered for goldfish at 20°C in this study. Temperature-related trends in water content have been quite variable with at least four patterns encountered : (1) a mid-temperature low (Prosser, _et al_., 1970); (2) stable values (Heinicke & Houston, 1965b); (3) an increase (Das, 1967); and (4) a slight but noticeable decrease (Houston, _et al_., 1970). Water content in the goldfish of the present study did not conform to any of these patterns, but exhibited highest values at the uppermost static temperature (30°C). In this study, both Mg^{2+} and Ca^{2+} of the goldfish were characterized by maxima at 25°C. In the carp (Houston, _et al_., 1970), both of these electrolytes increased with increasing acclimation temperature.

The above comments suggest that tissue water-electrolyte concentrations are variable with respect to temperature, and are characterized by few or no consistent trends. However, if all available data are plotted, it becomes clear that maximum values tend to occur in the mid-temperature range with muscle ion concentrations at the higher and lower temperatures being reduced. The present results are consistent with this pattern. The underlying basis for these features remain uncertain and will only be clarified when more detailed studies on temperature-related changes in flux ratios, urine losses, ion partitioning, metabolic pathways and related information becomes available. Nevertheless, it seems apparent that maximum muscle electrolyte levels are closely correlated with what, on the basis of growth studies in particular, appears to be an "optimum" temperature zone for the goldfish.

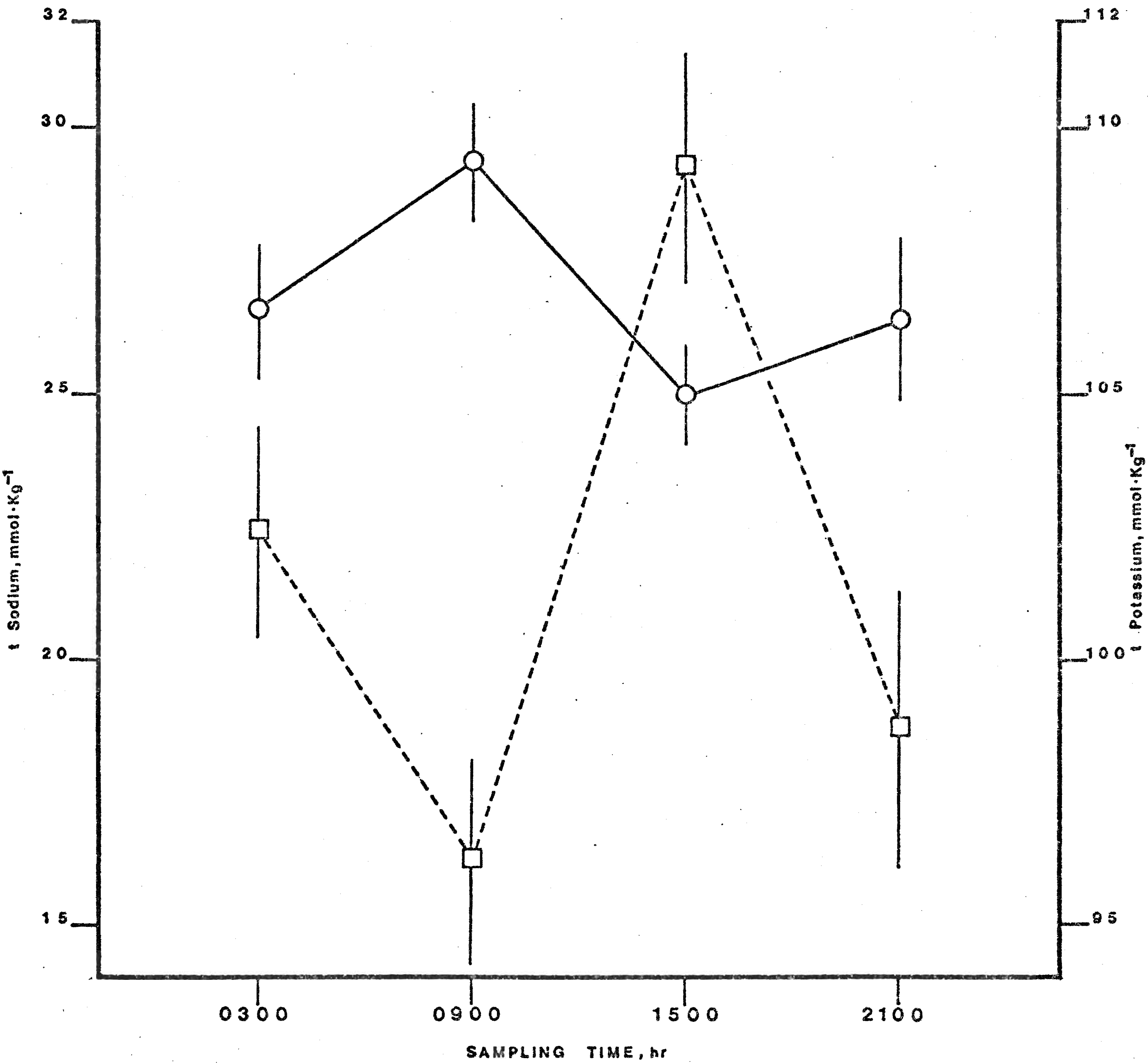
Although characterized by a number of procedural deficiencies (as noted earlier), the study of Toews and Hickman (1969) on rainbow trout, is important from the standpoint of muscle water electrolyte status in cycling and static temperature fish. Under static conditions, the following trends were observed: (1) water content dropped markedly at temperatures higher than 14°C; (2) Na^+ and Cl^- concentrations were stable with respect to temperature; and (3) K^+ levels exhibited a 21.6% increase from 8°C to 14°C. Both Na^+ and K^+ were characterized by maximum values at the mid-point temperature (25°C), while the Cl^- maximum corresponded to the lowest temperature (20°C). Water content in the goldfish actually peaked at the high temperature (30°C).

Under cycling conditions, rainbow trout were characterized by the following features : (1) water content, after an increase from 8°C to 10°C remained stable; (2) Cl^- levels were stable; (3) K^+ did not vary in any consistent fashion; and (4) Na^+ decreased substantially from 8°C to 16°C in the rising phase of the cycle. With the exception of minor, but significant differences, both Cl^- and water content were stable in the goldfish as well. Unlike the rainbow trout, however, goldfish Na^+ concentrations were relatively stable. K^+ levels were also different with peak levels associated with the high temperature of the cycle. The patterns shown by the latter two electrolytes are of importance in that their concentrations appeared to be inversely related in both investigations (see Figure 23).

The underlying basis for these fluctuations is still unknown and needs to be clarified. However, the differences seen between the two studies may be related to optimum temperature values 15°C for the rainbow trout and 25°C for the goldfish. It was shown earlier that, in the goldfish, maximum muscle concentrations correspond to the optimum temperature zone of this animal. The values in rainbow trout follow much the same pattern.

Differences in the cycled fish may be related to the temperature cycles themselves. Evidence for this can be obtained from thermal shock experiments (Houston, 1962; Hickman, *et al*., 1964; Heinicke & Houston, 1965 a, b; Reeves, *et al*., 1968). From such work, two important factors related to the application of thermal stresses have emerged: (1) in terms of changes in concentration and distribution, the rate at which a stress is imposed is critical; and (2) the overall magnitude of the applied stress is more important

Figure 23 : Relationship between muscle sodium (open circles) and potassium (open squares), ($mmol \cdot Kg^{-1}$) in cycling temperature goldfish at the four sampling periods of 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr. Symbols are centered on the mean with vertical bars representing 1 standard error of the mean.


32
30
25
20
15
112
110
105
100
95
↑ Sodium, mmol·Kg^{-1}
↑ Potassium, mmol·Kg^{-1}
0300
0900
1500
2100
SAMPLING TIME, hr

than the quality of the stress. With respect to these factors, this study and that of Toews and Hickman (1969), are clearly different. In the present investigation, temperature change was sinusoidal in nature with variations above and below the mid-point equal. Toews and Hickman (1969), on the other hand, applied a very rapid increase with unequal exposure to the high and low temperatures. In addition, the nature of the cycle was such that there was no equivalent fluctuation about a mid-point temperature.

When the present parameters were considered from the standpoint of cycling versus static thermal conditions, the following trends were noted : (1) Na^+ and Cl^- concentrations of the former group exceeded those of the latter with differences being significant for Cl^- but not so for Na^+; (2) with the exception of K^+ at 15:00 hr, Mg^{2+} at 03:00 hr and water content at 21:00 hr, all other ion parameters were significantly lower in cycling temperature animals than they were under the constant temperatures; and (3) both total cations and total cations + Cl^- exhibited the same pattern as outlined in point (2); however, the degree of significance was not as pronounced. The above relationships were obtained using static temperature values paralleling those of the cycle. If the overall static temperature readings were utilized, these relationships become less apparent and, in the case of water content, total cations and total cations +

Cl^- slightly altered (cycling temperature values corresponded to the lower range of the static readings).

The procedure utilized by Toews and Hickman (1969), was such that static temperature values corresponding to those of the cycle were obtained. Therefore, their results can be compared to the trends listed above. Both Na^+ and K^+ exhibited opposite patterns to those presently obtained, while Cl^- concentrations, unlike the goldfish, did not vary significantly from those under the cycling thermal regime. With the exception of the uppermost static temperatures, constant temperature values were similar to those of cycling temperature animals. This was in contrast to the pattern seen in goldfish.

These discrepancies can be attributed to the nature of the cycle utilized in each case. This feature has been previously discussed and need not be considered any further.

Principal Findings of the Study

1) Hematological parameters in cycling temperature fish were more diurnally stable and somewhat higher than those of the constant temperature groups. This suggests that under cycling temperature conditions, these fish may be responding to higher rather than lower temperatures of the cycle.

2) Erythrocytic composition under cycling temperature conditions was such as to promote reductions in hemoglobin-oxygen affinity and thus facilitate oxygen release at the cellular levels. Generally comparable, but much less pronounced, trends were observed in fishes held under constant temperature conditions. In each instance, these potentially-adaptive changes took the form of some combination of increases in cellular chloride and/or potassium coupled with decreases in magnesium and/or calcium and/or sodium.

3) As evidenced by the prevalence and magnitude of diurnal changes in plasma electrolyte levels it is apparent the animals acclimated to cycling temperatures, despite the fact that these place substantial stress upon water and electrolyte regulating systems, are capable of more precise control than are those held at constant temperature. In general, data on red cell and muscle corroborate this.

Significance of the Principal Findings

1) The stability of plasma ion composition in cycled as compared to constant temperature fishes and the differences seen in the concentrations of their principal plasma ions lead to two inferences. Earlier studies based on long-term maintenance under constant temperature conditions - circumstances which must be regarded as abnormal for many freshwater fishes - may provide an inadequate representation of iono-regulatory capability. The diurnal deviations in plasma composition characteristic of the constant, as compared to cycling temperature groups, suggest that these animals are, in some way, "programmed" for regulation under fluctuating temperatures. The information gained in this study provides no insights as to the mechanism(s) involved. The variabilities encountered under constant temperature conditions, however, suggest continuance of some cycle-compensating system despite the absence of appropriate (and, in a sense, "normal") thermal stimuli.

2) Under normoxic circumstances, goldfish exposed to both constant and cycling temperature conditions appear to modify red cell ion composition in a manner appropriate to reduced hemoglobin-oxygen affinity. This can be regarded as adaptive in the sense of establishing conditions in which oxygen release at the tissue level would be facilitated when oxygen demand is elevated.

3) In more general terms, the present data suggest that the goldfish appears to possess the ability to acclimate to a cycling thermal regime about its optimum temperature.

Literature Cited

Anthony, E.H. 1961. The oxygen capacity of the goldfish (Carassius auratus L.) blood in relation to thermal environment. J. Exp. Biol., 38 : 93-107.

Antonini, E., Wyman, J., Rossi-Fanelli A., and Caputo, A. 1962. Studies on the relationships between molecular and functional properties of hemoglobin. III. The influence of salts on the Bohr effect in human hemoglobin. J. Biol. Chem., 237 : 2773-2777.

Arnone, a. 1972. X-ray diffraction study of binding of 2, 3- dephosphoglycerate to human deoxyhaemoglobin. Nature (London), 237 : 146-149.

Bartlett, G.R. 1978a. Water soluble phosphates of fish red cells. Can. J. Zool., 56 : 870-877.

Bartlett, G.R. 1978b. Phosphates in red cells of two South American osteoglossids : Arapaima gigas and Osteoglossum bicirrhosum. Can. J. Zool., 56 : 878-881.

Bartlett, G.R. 1978c. Phosphates in red cells of two lung-fish, the South American, Lepidosiren paradoxa, and the African, Protopterus aethiopicus. Can. J. Zool., 56 : 882-886.

Beamish, F.W.H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. II Influence of weight and temperature on respiration of several species. Can. J. Zool., 42 : 177-188.

Beamish, F.W.H., and Mookherjii, P.S. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on the respiration of goldfish, Carassius auratus. L. Can. J. Zool., 42 : 161-175.

Benesch, R. and Benesch, R.E. 1967. The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. Biochem. Biophys. Res. Commun., 26 : 162-167.

Benesch, R., Benesch, R.E. and Yu, C.I. 1968. Reciprocal binding of oxygen and dephosphoglycerate by human hemoglobin. Proc. Natn. Acad. U.S.A., 59 : 526-532.

Benesch, R.E., R. Benesch and Yu, C.I. 1969. The oxygenation of hemoglobin in the presence of 2, 3-diphosphoglycerate. The effect of temperature, pH, ionic strength and hemoglobin concentration. Biochemistry, 8 : 2567-2571.

Bridges, D.W., Cech, J.J. and Pedro, D.N. 1976. Seasonal hematological changes in winter flounder, Pseudo-peuronectes americanus. Trans. Am. Fish. Soc., 105 : 596-600.

Biette, R.M. and Geen, G.H. 1980. Growth of underyearling sockeye salmon (Oncorhynchus nerka) under constant and cyclic temperatures in relation to live zooplankton ration size. Can. J. Fish. Aquat. Sci., 37 : 203-210.

Bruin, S.H., Rollema, H.S., Janssen, L.H.M. and Van Os, G.A.J. 1974. The interaction of chloride ions with human hemoglobin. Biochem. Biophys. Res. Comm., 58 : 210-215.

Brunori, M. 1975. Molecular adaptation to physiological requirements : the hemoglobin system of trout. Curr. Top. Cell. Regul., 9 : 1-39.

Bunn, H.F., Ransil, B.J. and Chao, A. 1971. The interaction between erythrocyte organic phosphates, magnesium ion and hemoglobin. J. Biol. Chem., 246 : 5273-5279.

Bygrave, L.L. 1967. Ionic environment and metabolic control. Nature, 214 : 667-671.

Byrne, J., Beamish, F.W., and Saunders, R.L. 1972. Influence of salinity, temperature and exercise on plasma osmolality and ionic concentration in Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can., 29 : 1217-1220.

Cameron, J.N. 1974. Evidence for the lack of bypass shunting in teleost gills. J. Fish. Res. Bd. Can., 31:211-213.

Cameron, J.N. 1976. Branchial ion uptake in Arctic grayling: resting values and effects of acid-base disturbance. J. Exp. Biol., 64 : 711-725.

Cameron, J.N. 1978. Chloride shifts in fish blood. J. Exp. Zool., 206 : 289-295.

Cameron, J.N. and Cech, J.J. 1970. Notes on the energy costs of gill ventilation in teleosts. Comp. Biochem. Physiol., 34 : 447-455.

Cameron, J.N. and Davis, J.C. 1970. Gas exchange in rainbow trout, (*Salmo gairdneri*) with a varying blood oxygen capacity. J. Fish. Res. Bd. Can., 27 : 1069-1085.

Catlett, R.H., and Millich, D.R. 1976. Intracellular and extracellular osmoregulation of temperature acclimated goldfish: *Carassius auratus*. Comp. Biochem. Physiol., 55A : 261-269.

Chanutin, A. and Curnish, R. 1967. Effect of organic and inorganic phosphate on the oxygen equilibrium of human erythrocytes. Arch. Biochem. Biophys., 121 : 96-102.

Christensen, H.N. 1975. Biological Transport (2nd edition). W.A. Benjamin, Reading, Massachusetts, U.S.A.

Dalmark, M. 1976. Chloride in the human erythrocyte. Prog. Biophys. Molec. Biol., 31 : 145-164.

Dame, R.F. and Vernberg, F.J. 1978. The influence of constant and cyclic acclimation temperatures on the metabolic rates of *Panopeus herbstii* and *Uca pugilator*. Biol. Bull., 154 : 188-197.

Das, A.B. 1967. Biochemical changes in tissues of goldfish acclimated to low and high temperatures. II. Synthesis of protein and RNA of subcellular fractions and tissue composition. Comp. Biochem. Physiol., 21 : 469-485.

Davis, J.C. 1972. An Infrared photographic technique useful for studying vascularization of fish gills. J. Fish. Res. Bd. Can., 29 : 109-111.

Davis, R. 1961. Carbonic Anhydrase. *In* The Enzymes V. (Boyer, P., Lardy, H. and Myrback, K. Eds.), pp.545-562. Academic Press. N.Y.

Denton, J.E., and Yousef, M.K. 1975. Seasonal changes in hematology of rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., 51A :151-153.

De Renzis, G. and Maetz, J. 1973. Studies on the mechanism of chloride absorption by the goldfish gill. Relation with acid-base regulation. J. Exp. Biol., 59 : 339-358.

DeSmet, W.H.O. 1978. A comparison of the electrophoretic haemoglobin patterns of the vertebrates. Acta. Zool. Pathol. Antverpiensia, 70 : 119-131.

DeWilde, M.A., and Houston, A.H. 1967. Haematological aspects of the thermoacclimatory process in the rainbow trout. J. Fish. Res. Bd. Can., 24 : 2267-2281.

Eaton, J.W. 1974. Oxygen affinity and environmental adaptation. Ann. N. Y. Acad. Sci., 241 : 491-497.

Eddy, F.B. 1971. Blood gas relationships in rainbow trout, Salmo gairdneri. J. Exp. Biol., 55 : 695-711.

Eddy, F.B. 1973. Oxygen dissociation curves of the blood of the tench, Tinca tinca. J. Exp. Biol., 58 : 281-293.

Evans, D.H. 1969. Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. J. Exp. Biol., 50 : 689-703.

Falkner, N.W., and Houston, A.H. 1966. Some hematological responses to sublethal thermal shock in the goldfish, Carassius auratus. Fish. Res. Bd.Can., 23: 1109-1120.

Feldmeth, C.R., Stone, E.A. and Brown, J.H. 1974. An increased scope for thermal tolerance upon acclimating pupfish (Cyprinodon) to cycling temperatures. J. Comp. Physiol., 89 : 39-44.

Fleming, W.R. 1974. Electrolyte Metabolism of Teleosts - Including Calcified Tissues. In Chemical Zoology, Vol. 8 : Deuterostomians, Cyclostomes and Fishes (Edited by Florkin, M. and Scheer, B.T.), Academic Press, N.Y.

Fletcher, G.L. 1975. The effects of capture, 'stress', and storage of whole blood on the red blood cells, plasma, proteins, glucose and electrolytes of the winter flounder (Pseudopleuronectes americanus). Can. J. Zool., 53 : 197-206.

Fry, F.E.J. 1947. Effects of the environment on animal activity. University of Toronto Stud., Biol. Ser., No. 55. Pub. Cent. Fish. Res. Lab., No. 58. pp. 1-62.

Fry, F.E.J. 1964. Animals in aquatic environments : Fishes In "Handbook of Physiology" (Am. Physiol. Soc., J. Field, ed.), Sec. 4, pp. 715-728. Williams & Wilkins, Baltimore, Maryland.

Fry, F.E.J., Brett, J.R., and Clawson, G.H. 1942. Lethal limits of temperature for young goldfish. Rev. Can. Biol., 1 : 50-56.

Fry, F.E.J., Hart, J.S. and Walker, K.F. 1946. Lethal temperature relations for a sample of young speckled trout, Salvelinus fontinalis. Univ. Toronto. Stud., Biol. Serv. No. 54, Pub. Ont. Fish. Res. Lab., 66 : 9-35.

Fyhn, U.E.H., Fyhn, H.J., Davis, B.J., Powers, D.A., Fink, W.L., and Garlick, R.L. 1979. Hemoglobin heterogeneity in Amazonian fishes. Comp. Biochem. Physiol., 62A : 39-66.

Gary-Bobo, C.M., and Solomon, A.K. 1968. Properties of hemoglobin solutions in red cells. J. Gen. Physiol., 52 : 825-853.

Geoghegan, W.D., and Poluhowich, J.J. 1974. The major erythrocytic organic phosphates of the American eel, Anguilla rostrata. Comp. Biochem. Physiol., 49B : 281-290.

Gillen, R.G., and Riggs, A. 1972. Structure and function of the hemoglobin of the carp, Cyprinus carpio. J. Biol. Chem., 247 L 6039-6046.

Gillen, R.G. and Riggs, A. 1977. The enhancement of the alkaline Bohr effect of some fish hemoglobins with adenosine triphosphate. Biochem. Biophys., 183 : 678-685.

Greaney, G.S., and Powers, D.A. 1978. Allosteric modifiers of fish hemoglobins : in vitro and in vivo studies of the effect of ambient oxygen and pH or erythrocyte ATP concentrations. J. Exp. Zool., 203 : 339 - 350.

Grigg, G.C. 1969. Temperature-induced changes in the oxygen equilibrium curve of the blood of the brown bullhead, Ictalurus nebulosus. Comp. Biochem. Physiol., 28 : 1203-1223.

Haswell, M.S., and Randall, D.J. 1976. Carbonic anhydrase inhibitor in trout plasma. Respir. Physiol., 28 : 1-16.

Haswell, M.S. and Randall, D.J. 1978. The pattern of carbon dioxide excretion in the rainbow trout, Salmo gairdneri. J. Exp. Biol., 72 : 17-24.

Hattingh, J. and Van Pletzen, A.J.J. 1974. The influence of capture and transportation on some blood parameters of freshwater fish. Comp. Biochem. Physiol., 49A: 607-609.

Heath, A.G. 1973. Ventilatory responses of teleost fish to exercise and thermal stress. Amer. Zool., 13: 491-503.

Heath, A.G., and Hughes, G.M. 1974. Cardiovascular and respiratory changes during heat stress in the rainbow trout (Salmo gairdneri). J. Exp. Biol., 59 : 323-338.

Heath, W.G. 1963. Thermoperiodism in sea-run cutthroat trout, (Salmo clarki clarki). Science, 142 : 486-488.

Heinicke, E., and Houston, A.H. 1965a. A note on water balance in goldfish during lethal heat shock. Can. J. Zool., 43 : 847-852.

Heinicke, E. and Houston, A.H. 1965b. Effect of thermal acclimation and sublethal heat shock on ionic regulation in goldfish. J. Fish. Res. Bd. Can., 22 : 1455-1476.

Henry, J.A.C. 1980. Effect of diurnal cycling temperatures on cardiovascular-ventilatory function in statically acclimated rainbow trout, Salmo gairdneri. M. Sc. Thesis, Brock University.

Henry, J.A., and Houston, A.H. 1979 (unpublished observations).

Hickman, C.P., McNable, R.A., Nelson, J.S. Van Breenan, E.D., and Comfort, D. 1964. Effect of cold acclimation on electrolyte distribution in rainbow trout, Salmo gairdneri. Can. J. Zool., 42 : 577-597.

Hickman, C.P., and Trump, B.F. 1969. The kidney. In Fish Physiology (Hoar, W. and Randall, D., Editors). Vol. I. Academic Press, New York, pp. 91-239.

Hoar, W.S. 1955. Seasonal variations in the resistance of goldfish to temperature. Trans. of the Roy. Soc. of Canada, Ser. III, Vol. 49, pp. 25-34.

Hoar, W.S. and Dorchester, J.E.C. 1949. The effect of dietary fat on the heat tolerance of goldfish, (Carassius auratus). Can. J. Research. D, 27 : 87-91.

Hoar, W.S., and Cottle, M.K. 1952. Dietary fat and temperature tolerance of goldfish. Can. J. Zool., 30 : 41-48.

Hoar, W.S. and Robertson, G.B. 1959. Temperature resistance of goldfish maintained under controlled photoperiods. Can. J. Zool., 37 : 419-428.

Hochachka, P. and Somero, G. 1973. Strategies of Biochemical Adaptation. W.B. Saunders Co., Philadelphia.

Hol, W.G.J., van Duijen, P.T., and Berendsen, H.J.C. 1978. The α-helix dipole and the properties of proteins. Nature (London), 273 : 443-446.

Holland, R.A.B. 1970. Factors determining the velocity of gas uptake by intracellular hemoglobin. In Blood Oxygenation. (Edited by D. Hershey), Plenum Press, New York. pp. 1-23.

Holmes, W.N. and Donaldson, E.M. 1969. The Body Compartments and the Distribution of Electrolytes. In Fish Physiology (Hoar, W. and Randall, D., Editors). Vol.I. Academic Press, N.Y. pp. 1-89.

Houston, A.H. 1962. Some observations on water balance in the goldfish, Carassius auratus L., during cold death. Can. J. Zool., 40 : 1169-1174.

Houston, A.H. 1973. Environmental temperature and the body fluid system of the teleost. In Responses of Fish to Environmental Changes. (Edited by W. Chavin). Charles C. Thomas, Springfield. pp. 87-162.

Houston, A.H. 1980. Thermal Requirements of Freshwater Fishes. Monogr. NRC. Assoc. Comm. Water Quality Criteria (in press).

Houston, A.H. and DeWilde, M.A. 1968. Thermoacclimatory variations in the haematology of the common carp, Cyprinus carpio. J. Exp. Biol., 49 : 71-81.

Houston, A.H., DeWilde, M.A. and Madden, J.A. 1969. Some physiological consequences of aortic catheterization in the brook trout, Salvelinus fontinalis. J. Fish. Res. Bd. Can., 26 : 1847-1856.

Houston, A.H. Madden, J.A., Woods, R.J. and Miles, H.M. 1971. Variations in the blood and tissue chemistry of brook trout, Salvelinus fontinalis, subsequent to handling, anaesthesia and surgery. J. Fish. Res. Bd. Can., 28 : 635-642.

Houston, A.H., and Cyr, D. 1974. Thermoacclimatory variation in the haemoglobin systems of goldfish (Carassius auratus) and rainbow trout (Salmo gairdneri). J. Exp. Biol., 61 : 455-461.

Houston, A.H., Mearow, K.M., and Smeda, J.S. 1976. Further observations upon the hemoglobin systems of thermally-acclimated freshwater teleosts : pumpkinseed (Lepomis gibbosus), white sucker (Catostomus commersoni), carp (Cyprinus carpio), goldfish (Carassius auratus) and carp goldfish hybrids. Comp. Biochem. Physiol., 54A : 267-273.

Houston, A.H. and Rupert, R. 1976. Immediate response of the hemoglobin system of the goldfish, Carassius auratus, to temperature change. Can. J. Zool., 10 : 1737-1741.

Houston, A.H. and McCarty, L.S. 1978. Carbonic anhydrase (acetazolamide-sensitive esterase) activity in the blood, gill and kidney of the thermally-acclimated rainbow trout, Salmo gairdneri. J. Exp. Biol., 78 : 15-27.

Houston, A.H., and Mearow, K.M. 1979. Temperature-related changes in the erythrocytic carbonic anhydrase (acetazolamide-sensitive esterase) activity of the goldfish, Carassius auratus. J. Exp. Biol., 78 : 255-264.

Houston, A.H. and Mearow, K.M. 1979b. Temperature-related changes in the ionic composition and (HCO_3^-)- and (Na^+/K^+)-ATPase activities of the rainbow trout erythrocyte. J. Exp. Zool. (Under review).

Houston, A.H. and Mearow, K.M. 1979. (Unpublished observations).

Houston, A.H. and Smeda, J.S. 1979. Thermoacclimatory changes in the ionic microenvironment of hemoglobin in the stenothermal rainbow trout (Salmo gairdneri) and eurythermal carp (Cyprinus carpio), J. Exp. Biol., 80 : 317-340.

Hughes, G.M. 1964. Fish respiratory homeostasis. Sym. Soc. Exp. Biol., 18 : 81-107.

Isaia, J. 1972. Comparative effects of temperature on the sodium and water permeabilities of the gills of a stenohaline freshwater fish (Carassius auratus) and stenohaline marine fish (Serranus scriba, Serranus cabrilla). J. Exp. Biol., 57 : 359-366.

Iuchi, I. and Yamagami, K. 1969. Electrophoretic pattern of larval haemoglobins of the salmonid fish, Salmo gairdneri irideus. Comp. Biochem. Physiol., 28 : 977-979.

Jampol, L. and Epstein, F. 1970. Sodium-potassium activated adenosine triphosphate and osmotic regulation by fishes. Am. J. Physiol., 218 : 607-611.

Johansen, K. 1971. Gas exchange and circulation in fishes. Ann. Rev. Physiol., 33 : 569-612.

Johansen, K., and Lenfant, C. 1972. A comparative approach to the adaptability of O_2-Hb affinity. in Oxygen Affinity of Hemoglobin and Red Cell Acid-Base Status (Ed. P Astrup and M. Rorth). Copenhagen, Academic Press.

Johansen, K., and Weber, R.E. 1976. On the adaptability of haemoglobin function to environmental conditions. In Perspectives in Experimental Biology. (Ed. P. Spencer Davies), pp. 212-234. Oxford, Pergamon Press.

Johansen-Sjobeck, M.L., Dave, G., Larsson, A., Lewander K. and Lidman, U. 1975. Metabolic and hematological effects of starvation in the European eel, Anguilla anguilla L. - II. Hematology. Comp. Biochem. Physiol., 52A : 431-434.

Jones, D.R. 1971. Theoretical analysis of factors which may limit the maximum oxygen uptake of fish : the oxygen cost of the cardiac and branchial pumps. J. Theor. Biol., 32 : 341-349.

Jones, D.R. and Randall, D.J. 1978. The respiratory and circulatory systems during exercise. In Fish Physiology (Eds. W.S. Hoar and D.J. Randall), Vol. 7, pp. 425-501, Academic Press, N.Y.

Kamra, S.K. 1966. Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (Gadus morhua). J. Fish. Res. Bod. Can., 23 : 975-982.

Kerstetter, T.H. and L.B. Kirschner. 1972. Active chloride transport by the gills of rainbow trout (Salmo gairdneri). J. Exp. Biol., 56 : 263-272.

Lagler, K.F., Bardach, J.E., and Miller, R.R. 1962. Ichthyology, pp. 497. John Wiley & Sons, Inc., New York.

Little, J.R.1964. Determination of water and electrolytes in tissue slices. Anat. Biochem., 7 : 87-95.

Lykkeboe, G., Johansen, K. and Maloiy, G.M.O. 1975. Functional properties of hemoglobins in the teleost, *Tilapia grahami*. J. Comp. Physiol., 104 : 1-11.

Mackay,W.C. 1974. Effect of temperature on osmotic and ionic regulation in goldfish, *Carassius auratus*. J. Comp. Physiol., 88 : 1-9.

Mackay, W.C., and Beatty, D.D. 1968. The effect of temperature on renal function in the white sucker fish, *Catostomus commersoni*. Comp. Biochem. Physiol., 26 : 235-245.

Maetz, J. 1971. Fish gills : mechanisms of salt transfer in freshwater and seawater. Phil. Trans. Soc. London, 262 : 209-249.

Maetz, J. 1972. Branchial sodium exchange and ammonia excretion in the goldfish, *Carassius auratus*. Effects of ammonia-loading and temperature changes. J. Exp. Biol., 56 : 601-620.

Maetz, J. 1974. Aspects of Adaption to Hypo-osmotic and Hyper-osmotic Environments. *In* "Biochemical and Ciophysical Perspectives in Marine Biology". Vol. 1. (Malins, D., and J. Sargent, Editors), pp. 1-167. Academic Press, New York.

Maetz, J. and F. Garcia-Romeu. 1964. Mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. II. Evidence for NH_4/Na^+ and HCO_3^-/Cl^- exchanges. J. Physiol., London, 47 : 1209-1227.

Manwell, C. 1957. Alkaline denaturation of hemoglobin of post-larval and adult *Scorpaenichthys marmoratus*. Science, 126 : 1175-1176.

Maren, J. 1967. Carbonic anhydrase in the animal kingdom. Fed. Proc., 26 : 1097-1103.

McCartney, J. 1976. Sodium-potassium dependent adenosine triphosphatase activity in gills and kidney of Atlantic salmon, *Salmo salar*. Comp. Biochem. Physiol., 53A : 351-353.

McCarty, L.S., and Houston, A.H. 1977. Na^+ : K^+ and HCO_3^--stimulated ATPase activities in the gills and kidneys of thermally-acclimated rainbow trout, Salmo gairdneri. Can. J. Zool., 55 : 704-712.

Mearow, K.M. 1979. Water and Electrolyte Content and Distribution in Tissues of Thermally-acclimated Rainbow Trout, Salmo gairdneri. M.Sc. Thesis, Brock University.

Mearow, K.M. 1976. Thermoacclimatory variations in hemoglobin systems of wild goldfish, Carassius auratus, carp Cyprinus carpio, and their natural hybrid. B.Sc. Thesis, Brock University, St. Catharines, Ontario.

Mearow, K.M. and Houston, A.H. 1979. (Unpublished observations).

Mied, P. and Powers, D.A. 1978. The hemoglobins of the killifish, Fundulus heteroclitus : separation, characterization and a model for the subunit composition. J. Biol. Chem., 253 : 3521-3528.

Motais, R., and Isaia, J. 1972. Temperature-dependence of permeability to water and to sodium of the gill epithelium of the eel, Anguilla anguilla. J. Exp. Biol., 56 : 587-600.

Munroe, V., and Poluhowich, J.J. 1974. Ionic composition of the plasma and whole blood of marine and fresh-water eels, Anguilla rostrata. Comp. Biochem. Physiol., 49A : 541-544.

Murphy, P.G.F., and Houston, A.H. 1977. Temperature, photo-period and water-electrolyte balance in the rainbow trout, Salmo gairdneri. Can. J. Zool., 55 : 1377-1388.

Natelson, S., and Natelson, E.A. 1978. Principles of Applied Clinical Chemistry : Chemical Background and Medical Applications. Vol. 2. The Erythrocyte : Chemical Composition and Metabolism. Plenum Press, N.Y.

Oikari, A., and Soivio, A. 1975. Influence of sampling methods and anaesthetization on various hematological parameters of several teleosts. Aquaculture, 6 : 171-180.

Perez, J.E. and Maclean, N. 1976. The haemoglobins of the fish, Sarotherodon mossambicus (Peters) : functional signif-icance and ontogenetic changes. J. Fish. Biol., 9 : 447-455.

Peterson, A.J. and Poluhowich, J.J. 1976. The effects of organic phosphates on the oxygenation behaviour of eel multiple hemoglobins. Comp. Biochem. Physiol., 55A : 351-354.

Phillips, A.M. 1969. Nutrition, Digestion and Energy Utilization. In Fish Physiology (Hoar, W. and Randall, D. Editors). Vol. I. Academic Press, N.Y. pp. 391-432.

Powers, D.A. 1972. Hemoglobin adaptation for fast and slow water habitats in Sympatric catostomid fishes. Science, 177 : 360-362.

Powers, D.A. 1974. Structure, function and molecular ecology of fish hemoglobins. Ann. N.Y. Acad. Sci., 241 : 472-490.

Powers, D.A., Martin, J.P., Garlick, R.L., Fyhn, H.J. and Fyhn, U.E.H. 1979. The effect of temperature on the oxygen equilibria of fish hemoglobins in relation to environmental thermal variability. Comp. Biochem. Physiol., 62A : 87-94.

Prosser, C.L. 1973. Comparative Animal Physiology. W.B. Saunders Co., Philadelphia.

Prosser, C.L., Mackay, W., and Kato, K. 1970. Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. Physiol. Zool., 43 : 81-89.

Rahn, H. 1966. Aquatic Gas Exchange : Theory. Respiration Physiology, Vol. 1. pp. 1-12, 1966.

Randall, D.J. 1970b. Gas exchange in fishes. In Fish Physiology. Vol. 4. (W.S. Hoar and D.J. Randall, Editors), Academic Press, New York, pp. 253-292.

Randall, D.J., Holston, G.F., and Stevens, E.D. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol., 46 : 339-348.

Randall, D.J., Baumgarten, D., and Malyusz, M. 1972. The relationship between gas and ion transfer across the gills of fishes. Comp. Biochem. Physiol., 41A : 629-637.

Ramirez-Munoz, J. 1968. Atomic Absorption Spectrophotometry. Elsevier Publishing Company, p. 269.

Reaves, R.S., Houston, A.H., and Madden, J.A. 1968. Environmental temperature and the body fluid system of freshwater teleosts. II. Ionic regulation in rainbow trout following abrupt thermal shock. Comp. Biochem. Physiol., 25 : 848-860.

Reeves, R.B. 1977. The interaction of body temperature and acid-base balance in ectothermic vertebrates. Ann. Revs. Physiol., 39 : 550-586.

Reutter, J.M. and Herdendorf, C.E. 1974. Laboratory estimates of the seasonal final temperature preferenda of some Lake Erie fish. Proc. 17th Conf. Great Lakes Res. 1974, pp. 59-67.

Richards, B.D. and Fromm, P.O. 1969. Patterns of blood flow through filaments and lamellae of isolated-perfused rainbow trout (*Salmo gairdneri*) gills. Comp. Biochem. Physiol., 29 : 1063-1070.

Riggs, A. 1970. Properties of fish hemoglobins. *In* Fish Physiology, Vol. 4 (W.S. Hoar and D.J. Randall, Editors), pp. 209-252, New York, Academic Press.

Riggs, A. 1976. Factors in the evolution of hemoglobin function. Fed. Proc. Fed. Am. Soc. Exp. Biol., 35 : 2115-2118.

Roberts, J.L. 1973. Effects of thermal stress on gill ventilation and heart rate in fishes. *In* Responses of Fish to Environmental Changes (Editor, W. Chavin), pp. 64-86, Charles C. Thomas, Springfield.

Rollema, H.S., DeBruin, S.H., Hanssen, L.H.M., and van Os, G.A.J. 1975. The effect of potassium chloride on the Bohr effect of human hemoglobin. J. Biol. Chem., 250 : 1333-1339.

Rossi-Fanelli, A., Antonini, E., and Caputo, A. 1961b. Studies on the relations between molecular and functional properties of hemoglobin. II. The effect of salts on oxygen equilibrium of human hemoglobin. J. Biol. Chem., 236 : 397-401.

Roy, A.W., and Johansen, P.H. 1970. The temperature selection of small hypophysectomized goldfish (*Carassius auratus L.*). Can. J. Zool., 48 : 323-326.

Scott, W.B., and Crossman, E.J. 1973. *Freshwater Fishes of Canada*. Fish. Res. Bd. Can., Bulletin 184, Ottawa, Canada.

Sharp, G.D. 1973. An electrophoretic study of hemoglobins of some scombroid fishes and related forms. Comp. Biochem. Physiol., 44B : 381-388.

Shelton, G. 1970. The regulation of breathing. In Fish Physiology. Vol. 4 (Eds. W.S. Hoar and D.J. Randall). pp. 293-359, Academic Press, N.Y.

Sidell, B.D., Wilson, R.F., Hazel, J., and Prosser, C.L. 1973. Time course of thermal acclimation in goldfish. J. Comp. Physiol., 84 : 119-127.

Smeda, J.S. 1978. M.Sc. Thesis. Thermoacclimatory Variations in the Microenvironment of Hemoglobin in the Rainbow Trout, Salmo gairdneri, and the carp, Cyprinus carpio. Brock University, St. Catharines, Ontario.

Smith, C.E. 1968. Hematological changes in coho salmon fed a folic acid deficient diet. J. Fish. Res. Bd. Can., 25 : 151-156.

Smith, M.W. 1970. Selective regulation of amino-acid transport by the intestine of the goldfish (Carassius auratus L.). Comp. Biochem. Physiol., 35 : 387-401.

Smith, M.W., Colombo, V.E., and Munn, E.A. 1968. Influence of temperature acclimatization on the ionic activation of goldfish intestinal adenosine triphosphatase. Biochem. J., 107 : 691-698.

Smith, M.W. and Ellory, J.C. 1971. Temperature-induced changes in sodium transport and Na^+/K^+- adenosine triphosphatase activity in the intestine of the goldfish (Carassius auratus L.). Comp. Biochem. Physiol., 39A : 209-218.

Soivio, A. and Oikari, A. 1976. Haematological effects of stress on a teleost, Esom lucius (L). J. Fish. Biol., 8 : 397-441.

Spieler, R.E., Noeske, T.A., DeVlaming, C., and Meier, A.H. 1977. Effects of thermocycles on body weight gain and gonadal growth in the goldfish, Carassius auratus. Trans. Am. Fish. Soc., 106(5) : 440-444.

Steen, J.B., and Kruysse. 1964. The respiratory function of teleostean gills. Comp. Biochem. Physiol., 12 : 127-142.

Steen, J.B., and Turitzin, S.N. 1968. The nature and biological significance of the pH difference across red cell membranes. Resp. Physiol., 5 : 234-242.

Tan, A.L., and Noble, R.W. 1973. The effect of inositol hexaphosphate on the allosteric properties of carp hemoglobin. J. Biol. Chem., 248 : 7412-7416.

Taylor, W., Houston, A.H., and Horgan, J.D. 1968. Development of a computer model simulating some aspects of the cardiovascular-respiratory dynamics of the salmonids fish. J. Exp. Biol., 49 : 477-494.

Threader, R.W. 1979. (Unpublished observations).

Toews, D.P., and Hickman, C.P. 1969. The effect of cycling temperatures on electrolyte balance in skeletal muscle and plasma of rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 29 : 905-918.

Torracca, A.M.V., Raschetti, R., Salvioli, R., Ricciardi, G., and Winterhalter, K.H. 1977. Modulation of the root effect in goldfish by ATP and GTP. Biochim. Biophys. Acta., 496 : 367-373.

Umminger, B.L. 1971. Patterns of osmoregulation in freshwater fishes at temperatures near freezing. Physiol. Zool., 44 : 20-27.

Vanstone, W.E., Roberts, E., and Tsuyuki, H. 1964. Changes in the multiple hemoglobin patterns of some Pacific salmon, genus Oncorhynchus during the parr-smolt transformation. Can. J. Physiol. Pharmacol., 42 : 697-703.

Van Vuren, J.H.J., and Hattingh, J. 1978. Seasonal changes in the haemoglobins of freshwater fish in their natural environment. Comp. Biochem. Physiol., 60 : 265-268.

Watson, T. 1978. Zinc effects on branchial adenosine triphosphatases and blood constituents of rainbow trout, Salmo gairdneri. Ph.D. Thesis, Department of Zoology, University of Guelph, Guelph, Ontario.

Weatherley, A.H. 1970. Effects of superabundant oxygen on thermal tolerance of goldfish. Biol. Bull., 139 : 229-238.

Weatherley, A.H. 1973. Effects of constant illumination and hyperoxia on thermal tolerance of goldfish. Comp. Biochem. Physiol., 45A : 891-894.

Weber, R.E., Lykkeboe, G., and Johansen, K. 1976. Physiological properties of eel haemoglobin: Hypoxic acclimation, phosphate effects and multiplicity. J. Exp. Biol., 64 : 75-88.

Weber, R.E., Wood, S.C., and Lomholt, J.P. 1976. Temperature acclimation and oxygen-binding properties of blood and multiple haemoglobins of rainbow trout. J. Exp. Biol., 65 : 333-345.

Weber, R.E. and Lykkeboe, G. 1978. Respiratory adaptations in carp blood : influences of hypoxia, red cell organic phosphates, divalent cations and CO_2 on hemoglobin-oxygen affinity. J. Comp. Physiol., 128 : 127-137.

Widdows, J. 1976. Physiological adaptation of Mytilus edulis to cyclic temperatures. J. Comp. Physiol., B. 105 : 115-128.

Wood, S.C., and Johansen, K. 1972. Adaptation to hypoxia by increased HbO_2 affinity and decreased red cell ATP concentration Nature, New Biol., 237 : 278-279.

Wood, S.C., Johansen, K. and Weber, R.E. 1975. Effects of ambient P_{O2} on hemoglobin-oxygen affinity and red cell ATP concentrations in a benthic fish, Pleuronectes platessa. Resp. Physiol., 25 : 259-267.

APPENDIX I

Natural History of the Goldfish*

Goldfish (*Carassius auratus*) can be classified according to the following detailed scheme (adapted from Lagler, Bardach and Miller (1962) and Scott and Crossman (1975)) :

Phylum		Chordata	
	Subphylum		Vertebrata
Class		Osteichthyes	
	Subclass		Actinopterygii
Order		Cypriniformes	
	Suborder		Cyprinoidei
Family		Cyprinidae	
Species		*Carassius auratus*	

The order Cypriniformes is second only to the Perciformes in both numbers and faunal dominance, thus making it one of the largest and most important assemblages of fish in the world. The aforementioned orders can also be distinguished by habitat preference, the former dominating freshwater, while the latter, the marine environment. At the family level, the Cyprinidae comprises the largest of all fish families with 275 genera and 1500 species. Its members are termed primary freshwater fishes due to their dependence

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* Adapted from Scott and Crossman (1973)

on freshwater and intolerance of salt water. In Canada, this family has 44 species, of which only three can be considered "exotic". The goldfish, the carp (Cyprinus carpio [Linnaeus]) and the tench (Tinca tinca [Linnaeus]) were all introduced to North America from Europe.

The goldfish, a species indigenous to eastern Asia, is believed to have its origin in China. For example, their appearance as pets has been recorded as early as the initial portion of the Sung Dynasty, approximately around the year 960. Their subsequent popularity has increased to the extent that selective breeding for specific morphological characteristics has resulted in the emergence of highly specialized strains. Today the "common" and "comet" varieties most closely resemble the original pond goldfish.

The end of the 17th century or the early portion of the 18th century marked the spread of these animals into Britain and continental Europe. Their introduction into North America is not definitely known although a goldfish farm in Maryland was reported by the year 1889. Presently, they are found widespread throughout the U.S.A. but in Canada they display a sporadic distribution. Populations of these fish have not been reported in the maritime provinces, Quebec, Manitoba or Saskatchewan. In both British Columbia and Alberta, only a very limited number of occurrences have been documented. Their presence in Ontario is comparatively more common with populations occurring in Lake St. Clair, the Detroit River and various parts of Lake Erie. In fact, in Southern Ontario (particularly in the Niagara Peninsula) small shallow ponds and lakes with rich supplies of aquatic plants possess abundant numbers of these fishes.

"Wild" populations of goldfish are not usually characterized by their ancestral gold colouring, but have reverted to an olive-green shade. Other individuals can also be found with patches of gold, white and/or black, and, in some cases, may be an almost translucent pearl-white colour. This predominance of non-gold "wild" fish may be a result of selective predation on the more highly coloured forms. Goldfish can freely hybridize with carp and, in so doing, produce progeny that are olive-to grey-green in colour. In the Niagara region, these latter forms are often more plentiful than the parental species.

Both the carp and the goldfish possess similar reproductive biologies as seen from the fact that inter-breeding between the two does take place. Spawning occurs in the spring with May and June being the most active periods in Southern Ontario, while in other areas it may occur as late as mid-August. During the early morning hours, adhesive eggs are released in warm, weedy shallows by females accompanied by two or more males. Hatching of the eggs is temperature-dependent but usually requires three or more days. Development within the eggs can take place over quite a broad temperature range.

The omnivorous goldfish feeds on a variety of food items. It consumes a diverse array of insects (both larvae and adults), molluscs, crustaceans, aquatic worms and aquatic vegetation.

Adult goldfish possess stout, thickset bodies, (body depth 28 to 34% of the total length) with average total lengths spanning the 127 to 254 mm range (5-10 inches). A broadly triangular head averaging 24-26% of the total body length has a short (25-37% of the head length), broad (inter-orbital width 35.7-42.8% of the head length) snout. The relatively small mouth is protrusible and lacks barbels. In general, fins are heavy, translucent and are usually the same colour as the body. Both the dorsal and anal fins are single in number, the former possessing an anterior spine and 17 (15-18) soft rays, the latter, one anterior spine and 5 or 6 soft rays. In each case, the anterior spine is serrated along the trailing edge. The caudal fin may be very ornamental but, in general, is broadly forked. Pelvic fins, short and broad in nature, are located in a thoracic position with 9 (8) soft rays. The pectorals, with 15 to 17 soft rays are also short and broad. Goldfish are covered with large, firmly attached cycloid scales that form a complete lateral line, 27 to 30 scales in length. Breeding males have fine nuptial tubercles on their opercles, back (sometimes) and pectoral fins (a few).

Carp and goldfish can be differentiated according to several morphologically distinct features. The two barbels located on the upper jaw of carp are totally lacking in goldfish. Goldfish possess 37-43 gill rakers and 27-30 lateral line scales, while the corresponding values for carp are 21-27 and 35-39 respectively. Vertebral counts also differ in the two species, 35 or 36 for the carp and 28 or 29 for goldfish. Goldfish possess pharyngeal teeth that are not molar-like, a feature commonly found in carp. Carp-goldfish hybrids typically display features intermediate to those of either parental stock. Only one pair of barbels (one or both of which may be rudimentary) can be found. 30-40 and 32-33 are the counts reported for gill rakers and lateral line scales respectively. In most cases, the hybrid more closely resembles the carp in general body shape, having a more elongated head.

The goldfish is widely used as an experimental animal and is often termed the aquatic counterpart of the laboratory rat and guinea pig. Several reasons exist for its abundant use in these situations. Propagation in adequate facilities is easily controlled by the manipulation of temperature-photoperiod conditions and/or hormone administration. Problems of maintenance are minimal due to a number of inherent characteristics. These fishes are the most eurythermal of the North American teleosts and can be held at temperatures ranging from $\sim 0^{\circ}C$ to $\sim 42^{\circ}C$. They also exhibit a high degree of respiratory independence. This is borne out by the fact that their oxygen consumption at $20^{\circ}C$ is not influenced by oxygen availability at concentrations greater than 2.5 mg $O_2 \cdot l^{-1}$. In most cases, the tolerance of goldfish to pollutant and other related stresses is more pronounced than that in similar species. In short, these features make the need for a continuous flow water supply non-compulsory.

APPENDIX II

APPENDIX
TABLE 1: A summary of hemoglobin content (gm•100 ml^{-1}) and packed cell volume (%) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods. Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE 20°C	25°C	30°C	25° ± 5°C
Hemoglobin Content (gm/100 ml)	03:00 hr	6.54± 0.336	8.47± 0.531	8.59± 0.362	8.10± 0.164
	09:00 hr	6.87± 0.449	6.75± 0.287	7.78± 0.621	8.81± 0.207
	15:00 hr	8.04± 0.382	7.58± 0.350	8.17± 0.407	8.26± 0.200
	21:00 hr	7.89± 0.325	7.31± 0.307	7.01± 0.420	8.42± 0.215
Packed Cell Volume (%)	03:00 hr	23.7± 1.13	28.5± 0.947	28.9± 1.11	29.3± 0.862
	09:00 hr	25.9± 1.38	25.4± 0.863	28.5± 1.15	29.6± 0.745
	15:00 hr	28.3± 1.25	29.7± 0.741	27.9± 1.12	29.5± 0.767
	21:00 hr	29.9± 0.994	28.6± 0.631	24.0± 0.764	29.3± 0.728

APPENDIX

TABLE 2 : A summary of plasma water ($Kg \cdot Kg^{-1}$) and electrolyte ($mmol \cdot l^{-1}$) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 03:00 hr, 09:00 hr 15:00 hr and 21:00 hr sampling periods.

Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C)			
		20°C	25°C	30°C	25° ± 5°C
Plasma Na^+ (mmol/l)	03:00 hr	133.34 ± 1.08	130.35 ± 1.23	133.52 ± 1.14	128.08 ± 1.53
	09:00 hr	135.65 ± 0.843	136.21 ± 1.19	134.41 ± 0.767	128.01 ± 2.39
	15:00 hr	135.22 ± 0.827	132.09 ± 1.02	130.28 ± 0.559	128.09 ± 2.38
	21:00 hr	136.18 ± 1.07	133.54 ± 1.36	132.00 ± 0.827	131.77 ± 1.41
Plasma K^+ (mmol/l)	03:00 hr	2.69 ± 0.126	2.61 ± 0.090	2.91 ± 0.104	2.68 ± 0.056
	09:00 hr	2.87 ± 0.082	2.90 ± 0.105	2.87 ± 0.078	2.80 ± 0.060
	15:00 hr	2.93 ± 0.073	3.24 ± 0.198	2.97 ± 0.112	2.84 ± 0.080
	21:00 hr	3.24 ± 0.080	3.08 ± 0.158	2.84 ± 0.089	2.77 ± 0.063
Plasma Mg^{2+} (mmol/l)	03:00 hr	0.88 ± 0.039	0.69 ± 0.032	0.99 ± 0.019	0.87 ± 0.032
	09:00 hr	0.92 ± 0.015	0.74 ± 0.045	1.03 ± 0.029	0.80 ± 0.057
	15:00 hr	0.94 ± 0.036	0.78 ± 0.045	0.97 ± 0.027	0.85 ± 0.041
	21:00 hr	0.91 ± 0.037	0.72 ± 0.044	1.04 ± 0.037	0.86 ± 0.056
Plasma Ca^{2+} (mmol/l)	03:00 hr	2.37 ± 0.087	2.17 ± 0.060	2.55 ± 0.073	2.27 ± 0.035
	09:00 hr	2.22 ± 0.044	2.27 ± 0.073	2.34 ± 0.053	2.31 ± 0.031
	15:00 hr	2.24 ± 0.094	2.44 ± 0.084	2.45 ± 0.090	2.61 ± 0.068
	21:00 hr	2.37 ± 0.070	2.06 ± 0.079	2.58 ± 0.166	2.29 ± 0.041
Plasma Cl^- (mmol/l)	03:00 hr	109.06 ± 1.31	111.47 ± 1.27	112.82 ± 0.916	102.06 ± 2.10
	09:00 hr	110.83 ± 0.904	112.42 ± 0.617	115.29 ± 1.24	103.49 ± 1.69
	15:00 hr	111.03 ± 1.22	113.24 ± 0.903	115.14 ± 0.610	104.41 ± 1.82
	21:00 hr	110.57 ± 0.810	113.06 ± 0.797	112.59 ± 0.910	104.76 ± 1.11
Plasma H_2O (Kg/kg)	03:00 hr	0.9629 ± 0.0020	0.9415 ± 0.0035	0.9449 ± 0.0045	0.9430 ± 0.0034
	09:00 hr	0.9477 ± 0.0033	0.9592 ± 0.0031	0.9432 ± 0.0036	0.9395 ± 0.0020
	15:00 hr	0.9421 ± 0.0036	0.9382 ± 0.0037	0.9574 ± 0.0023	0.9442 ± 0.0021
	21:00 hr	0.9443 ± 0.0021	0.9536 ± 0.0035	0.9520 ± 0.0024	0.9421 ± 0.0023

APPENDIX

TABLE 3 : A summary of erythrocytic water ($Kg \cdot Kg^{-1}$, packed cells) and electrolyte ($mmol \cdot 1^{-1}$, packed cells) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr sampling periods.

Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C) 20°C	25°C	30°C	25 ± 5°C
Erythrocytic Na^+ (mmol/l packed cells)	03:00 hr	12.08± 0.982	14.91± 1.33	15.86± 0.970	8.00± 0.444
	09:00 hr	11.88± 0.726	17.44± 0.847	16.55± 1.48	12.43± 0.926
	15:00 hr	10.69± 0.632	12.06± 0.913	17.75± 1.01	14.00± 0.770
	21:00 hr	11.18± 0.740	13.09± 1.33	16.93± 1.38	11.63± 1.09
Erythrocytic K^+ (mmol/l packed cells)	03:00 hr	101.21± 1.63	97.76± 1.37	101.65± 1.14	103.79± 0.955
	09:00 hr	103.85± 1.01	91.92± 0.952	100.63± 1.56	101.44± 0.584
	15:00 hr	103.61± 0.851	93.20± 1.17	98.15± 1.11	100.41± 0.836
	21:00 hr	98.82± 1.35	95.96± 2.08	99.63± 1.04	101.23± 1.18
Erythrocytic Mg^{2+} (mmol/l packed cells)	03:00 hr	8.00± 0.143	7.75± 0.610	6.57± 0.182	7.38± 0.230
	09:00 hr	7.76± 0.234	6.86± 0.201	6.57± 0.164	7.45± 0.127
	15:00 hr	8.16± 0.325	6.86± 0.201	6.70± 0.169	7.29± 0.187
	21:00 hr	7.76± 0.221	7.00± 0.140	6.97± 0.201	7.60± 0.135
Erythrocytic Ca^{2+} (mmol/l packed cells)	03:00 hr	0.372± 0.0388	0.229± 0.0196	0.088± 0.0097	0.100± 0.0071
	09:00 hr	0.322± 0.0158	0.162± 0.0231	0.084± 0.0106	0.095± 0.0040
	15:00 hr	0.293± 0.008	0.179± 0.0422	0.099± 0.0215	0.069± 0.0041
	21:00 hr	0.315± 0.0068	0.227± 0.0305	0.098± 0.0196	0.097± 0.0091
Erythrocytic Cl^- (mmol/l packed cells)	03:00 hr	67.65± 1.81	69.14± 2.84	76.06± 1.12	71.69± 1.01
	09:00 hr	72.93± 1.31	76.75± 1.64	79.54± 1.05	72.50± 1.27
	15:00 hr	78.60± 1.78	76.28± 1.68	78.29± 0.903	74.08± 1.07
	21:00 hr	75.64± 1.81	77.49± 1.48	77.91± 2.12	71.33± 1.15
Erythrocytic H_2O content (Kg/Kg packed cells)	03:00 hr	0.6908± 0.0095	0.6891± 0.0086	0.7014± 0.0086	0.6905± 0.0063
	09:00 hr	0.6879± 0.0153	0.6949± 0.0074	0.6810± 0.0082	0.7011± 0.0113
	15:00 hr	0.6965± 0.0129	0.6959± 0.0067	0.7043± 0.0045	0.7148± 0.0049
	21:00 hr	0.7032± 0.0097	0.6837± 0.0106	0.6940± 0.0111	0.6795± 0.0111

APPENDIX

TABLE 4 : A summary of erythrocytic electrolyte (mmol·l^{-1}, cell H_2O) levels in goldfish acclimated to 20 °C, 25 °C, 30 °C and a cycling temperature of 25°± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods.

Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C) 20°C	25°C	30°C	25± 5°C
Na^+ Erythrocytic Electrolytes (mmol/l cell H_2O)	03:00 hr	17.57± 1.44	21.52± 2.05	23.62± 2.93	11.61± 1.28
	09:00 hr	17.92± 1.17	25.26± 1.25	23.95± 2.00	17.83± 1.38
	15:00 hr	15.62± 1.04	18.03± 1.38	25.24± 1.49	19.61± 1.10
	21:00 hr	15.80± 1.08	19.32± 1.96	23.84± 1.70	16.40± 1.54
K^+ Erythrocytic Electrolytes (mmol/l cell H_2O)	03:00 hr	147.44± 2.96	140.64± 1.56	145.26± 4.99	150.60± 2.19
	09:00 hr	152.88± 3.62	132.90± 1.65	149.71± 3.13	144.01± 1.47
	15:00 hr	148.76± 3.55	134.26± 1.62	139.31± 1.70	140.57± 1.57
	21:00 hr	137.99± 2.71	141.06± 3.94	144.24± 2.42	149.12± 1.68
Mg^{2+} Erythrocytic Electrolytes (mmol/l cell H_2O)	03:00 hr	11.46± 0.25	11.11± 0.41	9.37± 0.27	10.71± 0.36
	09:00 hr	11.65± 0.37	9.96± 0.28	9.59± 0.28	10.68± 0.22
	15:00 hr	11.91± 0.54	9.88± 0.28	9.48± 0.22	10.07± 0.31
	21:00 hr	11.14± 0.34	10.28± 0.20	9.92± 0.25	11.31± 0.24
Ca^{2+} Erythrocytic Electrolytes (mmol/l cell H_2O)	03:00 hr	0.54± 0.055	0.33± 0,032	0.13± 0.014	0.15± 0.010
	09:00 hr	0.50± 0.034	0.23± 0.033	0.12± 0.015	0.14± 0.006
	15:00 hr	0.43± 0.020	0.26± 0.061	0.16± 0.035	0.096± 0.006
	21:00 hr	0.44± 0.012	0.34± 0.045	0.16± 0.030	0.14± 0.014
Cl^- Erythrocytic Electrolytes (mmol/l cell H_2O	03:00 hr	97.93± 2.77	100.13± 4.84	107.52± 1.49	102.72± 2.21
	09:00 hr	105.10± 3.86	110.51± 2.32	114.90± 1.18	103.55± 2.01
	15:00 hr	111.53± 4.13	111.22± 2.28	111.76± 1.48	104.53± 1.76
	21:00	106.24± 2.96	115.40± 1.84	111.49± 3.44	106.39± 2.56

APPENDIX

TABLE 5 : A summary of ion : hemoglobin ratios (mmol·l^{-1}, packed cells/mmol·l^{-1}, packed cells) in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods.

Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C) 20°C	25°C	30°C	25 ± 5°C
Na^+/Hb Ratio (mmol/l pc/mmol/l pc)	03:00 hr	2.73 ± 0.224	3.27 ± 0.297	3.69 ± 0.263	1.84 ± 0.095
	09:00 hr	2.92 ± 0.220	4.32 ± 0.344	3.72 ± 0.285	2.98 ± 0.251
	15:00 hr	2.48 ± 0.168	3.19 ± 0.269	4.27 ± 0.227	3.41 ± 0.179
	21:00 hr	2.73 ± 0.188	3.49 ± 0.371	3.65 ± 0.272	2.71 ± 0.264
K^+ Hb Ratio (mmol/l pc/mmol/l pc)	03.00 hr	23.71 ± 0.534	21.72 ± 0.966	22.65 ± 0.750	24.23 ± 0.547
	09:00 hr	25.38 ± 0.486	23.31 ± 1.14	24.79 ± 0.933	23.32 ± 0.324
	15:00 hr	23.82 ± 0.600	23.52 ± 0.700	22.78 ± 0.745	24.29 ± 0.289
	21:00 hr	23.65 ± 0.489	24.94 ± 0.457	22.65 ± 0.687	23.22 ± 0.401
Mg^{2+}/Hb Ratio (mmol/l pc/mmol/l pc)	03:00 hr	1.82 ± 0.039	1.77 ± 0.125	1.49 ± 0.040	1.69 ± 0.061
	09:00 hr	1.89 ± 0.078	1.66 ± 0.123	1.57 ± 0.063	1.71 ± 0.036
	15:00 hr	1.92 ± 0.087	1.76 ± 0.095	1.58 ± 0.063	1.76 ± 0.055
	21:00 hr	1.86 ± 0.062	1.86 ± 0.081	1.59 ± 0.053	1.78 ± 0.026
Ca^{2+}/ Hb Ratio (mmol/l pc/mmol/l pc)	03:00 hr	0.084 ± 0.0098	0.053 ± 0.0069	0.020 ± 0.0021	0.024 ± 0.0020
	09:00 hr	0.082 ± 0.0059	0.039 ± 0.0073	0.021 ± 0.0026	0.022 ± 0.0012
	15:00 hr	0.068 ± 0.0029	0.052 ± 0.0131	0.033 ± 0.0079	0.017 ± 0.0010
	21:00 hr	0.077 ± 0.0030	0.063 ± 0.0096	0.022 ± 0.0039	0.022 ± 0.0019
Cl^-/ Hb Ratio (mmol/l pc/mmol/l pc)	03:00 hr	16.15 ± 0.546	15.38 ± 1.16	17.15 ± 0.376	16.41 ± 0.502
	09:00 hr	17.57 ± 0.596	19.74 ± 1.17	19.28 ± 0.637	16.16 ± 0.378
	15:00 hr	18.26 ± 0.576	19.19 ± 0.915	18.72 ± 0.465	18.27 ± 0.406
	21:00 hr	18.23 ± 0.580	20.40 ± 1.04	17.69 ± 0.833	16.56 ± 0.495

APPENDIX TABLE 6 : A summary of muscle water ($Kg \cdot Kg^{-1}$) and electrolyte ($mmol \cdot Kg^{-1}$) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25°± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods.

Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C) 20°C	25°C	30°C	25 ± 5°C
Tissue Na^+ (mmol/Kg)	03:00 hr	26.17± 1.32	25.01± 1.90	24.33± 1.09	26.56± 1.24
	09:00 hr	21.78± 0.872	28.78± 1.41	25.90± 1.16	29.36± 1.09
	15:00 hr	19.55± 0.672	24.19± 2.21	23.93± 1.17	24.99± 0.928
	21:00 hr	20.00± 0.578	27.74± 1.53	22.70± 0.987	26.40± 1.49
Tissue K^+ (mmol/Kg)	03:00 hr	110.36± 2.19	109.11± 2.45	106.15± 2.08	102.41± 1.97
	09:00 hr	106.01± 3.43	113.19± 1.86	104.96± 1.81	96.19± 1.89
	15:00 hr	105.69± 2.37	113.73± 1.51	107.09± 1.63	109.26± 2.16
	21:00 hr	106.21± 2.82	109.47± 6.56	106.41± 5.24	98.70± 2.63
Tissue Mg^{2+}(mmol/Kg)	03:00 hr	12.10± 0.495	14.20± 0.351	13.67± 0.266	12.62± 0.198
	09:00 hr	13.11± 0.270	14.05± 0.351	13.63± 0.217	12.22± 0.166
	15:00 hr	13.32± 0.365	14.59± 0.284	13.52± 0.332	12.30± 0.170
	21:00 hr	13.48± 0.319	14.65± 0.327	13.74± 0.273	12.93± 0.190
Tissue Ca^{2+}(mmol/Kg)	03:00 hr	7.18± 0.671	8.33± 1.21	8.33± 1.00	3.19± 0.199
	09:00 hr	4.18± 0.340	11.37± 1.47	8.25± 0.968	2.95± 0.242
	15:00 hr	4.05± 0.340	10.53± 1.53	9.95± 0.729	4.22± 0.412
	21:00 hr	4.28± 0.468	11.31± 1.19	8.46± 0.837	3.65± 0.347
Tissue Cl^- (mmol/Kg)	03:00 hr	12.94± 0.642	12.01± 0.561	11.42± 0.526	13.14± 0.432
	09:00 hr	13.85± 0.595	12.25± 0.426	12.95± 0.691	14.53± 0.389
	15:00 hr	12.39± 0.489	10.67± 0.411	11.76± 0.659	14.01± 0.503
	21:00 hr	12.31± 0.495	11.95± 0.454	11.74± 0.597	13.40± 0.460
Tissue H_2O (kg/kg)	03:00 hr	0.8010± 0.0038	0.7803± 0.0036	0.7955± 0.0016	0.7859± 0.0018
	09:00 hr	0.7852± 0.0019	0.7954± 0.0022	0.7965± 0.0034	0.7856± 0.0037
	15:00 hr	0.7865± 0.0018	0.7848± 0.0029	0.7996± 0.0030	0.7895± 0.0013
	21:00 hr	0.7832± 0.0021	0.7871± 0.0022	0.7959± 0.0021	0.7837± 0.0026

APPENDIX

TABLE 7 : A summary of plasma total cation ($mmol \cdot l^{-1}$) and total cation + Cl^- ($mmol \cdot l^{-1}$) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25° ± 5°C at the 0300 hr, 0900 hr, 1500 hr and 2100 hr sampling periods.
Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C) 20°C	25°C	30°C	25° ± 5°C
Total Plasma Cations (mmol/l)	03:00 hr	139.63 ± 1.12	135.90 ± 1.32	139.76 ± 1.18	133.98 ± 1.55
	09:00 hr	141.71 ± 0.90	141.97 ± 1.17	140.78 ± 0.72	134.07 ± 2.41
	15:00 hr	141.55 ± 0.82	137.47 ± 0.92	136.91 ± 0.94	134.43 ± 2.47
	21:00 hr	142.12 ± 1.20	139.60 ± 1.37	138.52 ± 0.93	137.86 ± 1.47
Total Plasma Cations + Cl^- (mmol/l)	03:00 hr	248.34 ± 2.18	246.92 ± 1.49	252.58 ± 1.40	233.66 ± 4.01
	09:00 hr	253.81 ± 1.39	255.87 ± 1.02	256.65 ± 1.68	241.03 ± 3.07
	15:00 hr	254.46 ± 1.24	250.41 ± 1.41	253.19 ± 1.16	236.84 ± 4.32
	21:00 hr	252.15 ± 1.60	252.44 ± 1.38	252.74 ± 1.20	243.69 ± 2.13

APPENDIX

TABLE 8 : A summary of erythrocytic total cation ($mmol \cdot l^{-1}$, cell H_2O) and total cation + Cl^- ($mmol \cdot l^{-1}$, cell H_2O) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25°± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods. Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C)			
		20°C	25°C	30°C	25°± 5°C
Total Erythro-cytic Cations (mmol/l cell H_2O)	03:00 hr	175.25± 3.56	169.50± 1.39	181.64± 2.45	174.33± 2.61
	09:00 hr	179.80± 3.40	165.64± 2.97	182.32± 2.40	174.24± 1.86
	15:00 hr	179.80± 5.30	162.28± 3.08	173.04± 2.43	170.03± 2.44
	21:00 hr	166.98± 3.91	169.49± 4.34	174.31± 2.68	177.36± 1.92
Total Erythro-cytic Cations + Cl^- (mmol/l cell H_2O)	03:00 hr	281.53± 4.33	271.41± 7.52	290.34± 2.77	276.90± 4.77
	09:00 hr	283.90± 6.01	277.43± 4.24	298.52± 3.32	279.88± 3.42
	15:00 hr	293.55± 10.37	276.10± 4.43	285.80± 3.20	274.56± 3.80
	21:00 hr	276.02± 6.58	287.41± 4.75	286.26± 5.52	281.46± 4.00

TABLE 9 : A summary of muscle total cation ($mmol \cdot Kg^{-1}$) and total cation + Cl^- ($mmol \cdot Kg^{-1}$) levels in goldfish acclimated to 20°C, 25°C, 30°C and a cycling temperature of 25°± 5°C at the 03:00 hr, 09:00 hr, 15:00 hr and 21:00 hr sampling periods. Reported as the mean ± 1 standard error of the mean.

PARAMETER	SAMPLING TIME	TEMPERATURE (°C)			
		20°C	25°C	30°C	25°± 5°C
Total Tissue Cations (mmol/kg)	03:00 hr	140.09± 7.09	151.99± 3.39	152.63± 2.69	143.08± 3.18
	09:00 hr	141.97± 4.39	163.43± 2.26	152.49± 2.59	140.86± 1.38
	15:00 hr	142.37± 3.36	159.99± 2.96	154.25± 1.52	149.74± 2.32
	21:00 hr	144.90± 3.58	166.22± 4.54	153.62± 2.32	142.63± 2.42
Total Tissue Cations + Cl^- (mmol/kg)	03:00 hr	156.25± 6.52	164.17± 3.19	164.51± 2.53	157.11± 2.72
	09:00 hr	155.83± 4.02	176.07± 2.17	165.75± 2.62	155.43± 1.16
	15:00 hr	153.12± 3.40	170.78± 3.13	168.19± 1.59	164.37± 2.55
	21:00 hr	157.22± 3.27	178.62± 4.52	164.14± 2.44	156.02± 2.62

APPENDIX III

APPENDIX

TABLE 10 : Length, weight, hemoglobin content and packed cell volume raw data for goldfish acclimated to 20°C.

SAMPLING TIME	CODE NO	FORK LENGTH (cm)	WEIGHT (gm)	Hb CONTENT (gm/100 ml)	PCV (%)
03:00 h	203	9.4	14.90	2.94	13.9
	204	9.0	12.10	6.57	28.7
	2015	8.0	9.25	6.76	26.3
	2016	8.9	11.60	4.95	16.3
	2021	11.2	22.61	5.33	22.9
	2022	12.7	34.40	7.60	26.7
	2023	15.9	74.31	8.05	27.3
	2024	15.1	58.41	7.32	23.7
	2025	14.4	57.62	7.63	25.1
	2026	15.4	77.97	7.74	26.2
	2027	16.4	86.62	9.21	33.9
	2028	11.5	24.47	5.41	21.2
	2029	12.1	27.33	6.04	23.9
	2030	14.8	68.76	7.64	26.7
	2031	12.2	27.66	5.83	18.7
	2032	11.1	27.57	6.65	24.8
	2033	12.3	28.40	5.83	18.3
	2034	9.7	17.66	1.65	9.0
	2035	12.3	29.45	6.22	21.6
09:00 h	205	8.9	12.90	5.96	24.7
	206	9.1	13.80	7.40	32.0
	2017	8.9	14.27	3.48	8.3
	2018	8.3	10.05	7.53	27.2
	2036	12.8	33.22	6.74	21.7
	2037	12.7	36.78	8.06	26.2
	2038	11.0	26.94	4.30	17.0
	2039	10.6	23.98	8.60	30.1
	2040	13.4	31.49	5.39	21.1
	2041	12.8	35.87	4.15	15.6
	2047	12.1	41.18	5.44	21.2
	2048	10.7	25.77	7.43	25.8
	2049	12.7	37.71	10.40	30.4
	2050	11.8	34.60	7.78	30.0
	2051	11.7	30.65	5.16	20.3
	2052	12.3	39.53	8.61	31.9
	2070	10.8	26.06	8.87	35.5
	2071	10.8	25.79	8.39	30.1
15:00 h	207	9.1	14.60	6.09	18.7
	208	8.7	12.10	3.60	14.1
	2011	9.0	13.50	8.64	31.9
	2012	8.5	10.70	5.43	20.0
	2019	9.0	10.40	7.77	26.8
	2020	8.3	11.30	8.96	34.5
	2053	12.1	30.99	8.06	29.4
	2054	11.8	40.83	10.56	36.3
	2055	11.7	28.08	8.57	27.3
	2056	12.0	35.77	7.82	31.0
	2057	12.5	33.04	8.50	28.3
	2063	10.8	26.06	7.07	26.1
	2064	10.4	24.29	8.48	28.5
	2065	11.9	35.20	9.20	32.6
	2066	11.1	37.60	10.97	35.3
	2067	11.2	32.81	8.68	28.9
	2072	10.8	27.67	6.55	24.2
	2073	10.7	25.07	5.36	21.3
21:00 h	201	10.1	16.00	6.40	25.3
	202	9.2	14.50	7.71	28.1
	209	9.2	14.60	9.58	33.9
	2010	8.8	12.90	7.92	32.1
	2013	9.1	13.20	6.76	28.9
	2014	8.1	10.90	7.71	31.2
	2042	12.0	40.20	5.89	16.1
	2043	12.1	28.47	5.74	19.8
	2044	11.3	32.84	10.30	35.3
	2045	11.1	31.40	6.98	25.2
	2046	10.8	24.55	9.22	28.0
	2058	11.5	31.52	8.30	30.8
	2059	11.5	37.19	8.45	33.1
	2060	11.4	29.79	8.06	29.6
	2061	11.7	39.86	8.89	30.8
	2062	11.7	38.42	10.05	37.1
	2068	11.2	30.40	6.26	29.5
	2069	11.9	32.79	7.83	29.5

APPENDIX

TABLE 11 : Length, weight hemoglobin content and packed cell volume raw data for goldfish acclimated to 25°C.

SAMPLING TIME	CODE NO	FORK LENGTH (cm)	WEIGHT (gm)	Hb CONTENT (gm/100 ml)	PCV (%)
03:00 h	254	9.4	16.90	6.06	27.4
	255	8.8	14.40	4.79	30.1
	256	10.05	18.90	8.09	20.8
	2522	8.7	12.65	6.38	27.6
	2523	8.8	13.85	5.92	25.5
	2524	8.7	10.80	6.35	27.5
	2535	12.9	38.69	9.75	30.5
	2536	11.2	25.65	10.85	33.1
	2537	12.8	37.17	10.14	38.3
	2564	15.3	77.94	7.49	24.5
	2565	14.6	71.80	8.03	24.4
	2566	13.5	52.58	8.11	26.7
	2567	11.2	32.52	8.07	27.0
	2568	13.1	54.65	9.56	31.4
	2569	10.8	28.46	10.21	31.2
	2570	14.3	69.67	12.47	35.1
	2571	13.3	53.67	11.64	32.8
09:00 h	257	9.1	14.20	7.05	34.7
	258	9.1	15.00	6.91	31.4
	259	8.6	10.45	5.41	24.0
	2525	8.2	10.50	4.72	24.1
	2526	8.1	9.90	6.22	29.8
	2527	8.7	12.45	6.55	29.2
	2538	10.6	24.05	7.18	27.9
	2539	12.3	32.46	8.00	24.0
	2540	12.5	30.29	7.26	20.2
	2544	12.7	39.94	7.11	23.8
	2545	12.4	36.90	7.43	26.0
	2546	13.8	45.82	7.46	25.6
	2547	12.8	36.81	6.80	25.6
	2548	15.9	72.04	8.34	23.2
	2549	11.8	29.21	7.44	25.1
	2550	10.9	20.16	4.29	19.8
	2551	11.6	19.65	4.35	18.8
	2552	12.6	35.51	8.66	30.8
	2553	11.7	26.45	7.15	28.1
15:00 h	2510	8.4	10.55	6.55	28.3
	2511	9.3	14.40	4.28	20.4
	2512	9.1	13.40	8.59	34.5
	2516	8.4	11.25	7.33	26.3
	2517	9.1	15.10	7.39	32.5
	2518	8.5	9.42	5.34	26.5
	2528	8.8	11.60	6.91	30.5
	2529	8.2	9.80	7.59	34.3
	2530	11.0	27.54	7.84	25.4
	2531	12.3	34.50	12.21	40.9
	2541	12.5	36.32	10.49	33.7
	2542	11.9	30.80	9.48	31.5
	2543	13.7	53.52	9.05	30.7
	2554	14.6	67.96	7.58	27.3
	2555	13.6	49.30	7.05	27.0
	2556	13.3	49.14	7.65	27.9
	2557	12.8	47.59	7.90	29.2
	2558	13.7	52.90	7.76	29.3
21:00 h	251	10.0	19.40	8.83	38.1
	252	9.2	11.00	5.49	17.9
	253	8.9	13.90	5.56	27.2
	2513	9.8	16.10	6.20	25.2
	2514	8.7	12.97	6.91	28.4
	2515	9.2	13.80	7.63	31.1
	2519	8.0	10.10	5.83	28.0
	2520	9.8	17.45	6.74	31.0
	2521	8.5	9.75	5.57	26.8
	2532	13.9	50.98	9.32	33.7
	2533	10.9	24.13	8.74	30.8
	2534	11.2	27.12	7.22	27.2
	2559	11.2	25.90	7.76	28.0
	2560	13.3	54.85	7.80	25.2
	2561	13.9	58.87	7.57	26.3
	2562	12.6	44.07	8.86	30.7
	2563	12.7	41.65	8.22	28.9

APPENDIX

TABLE 12 : Length, weight, hemoglobin content and packed cell volume raw data for goldfish acclimated to 30°C.

SAMPLING TIME	CODE NO	FORK LENGTH (cm)	WEIGHT (gm)	Hb CONTENT (gm/100ml)	PCV (%)
03:00 h	3011	10.5	27.91	9.65	29.1
	3012	10.7	21.83	10.73	31.6
	3013	11.7	22.50	9.75	31.9
	3014	12.9	33.44	9.24	25.2
	3015	12.1	31.68	13.37	39.8
	3032	12.5	37.60	8.94	32.0
	3033	12.4	35.79	6.76	20.9
	3034	13.4	40.63	10.18	35.7
	3035	10.1	18.49	9.84	35.0
	3059	11.3	32.52	6.40	24.1
	3060	12.0	36.00	7.69	27.6
	3061	12.8	42.84	8.24	29.8
	3062	16.5	75.98	7.61	25.5
	3063	12.3	38.33	7.32	28.4
	3064	12.7	42.77	7.87	28.4
09:00 h	3016	10.9	19.61	3.10	14.4
	3017	11.4	26.33	10.03	31.0
	3018	10.9	19.01	9.70	34.1
	3019	11.5	28.43	9.75	31.8
	2036	10.6	19.22	4.47	22.9
	3037	10.8	21.95	8.49	32.1
	3038	12.7	36.68	11.60	42.2
	3039	12.7	22.20	9.69	30.9
	3040	11.6	27.48	10.40	34.0
	3065	15.0	66.20	6.62	25.1
	3066	13.5	44.82	6.73	25.2
	3067	12.7	41.70	6.51	23.8
	3068	13.0	41.58	5.63	23.3
	3069	12.9	39.00	7.18	29.6
	3070	11.9	39.37	6.84	26.6
15:00 h	301	11.7	31.58	8.73	26.4
	302	11.8	29.85	5.70	18.8
	303	12.2	32.65	11.38	33.1
	304	13.3	34.72	10.68	32.8
	305	12.8	32.22	11.51	35.7
	3020	10.7	15.40	7.19	29.5
	3021	11.4	25.53	8.77	28.7
	3022	12.2	26.74	8.31	27.3
	3023	11.2	17.08	7.10	30.5
	3024	13.1	43.46	9.16	29.3
	3025	10.1	19.04	7.13	29.1
	3026	13.3	43.45	3.60	14.3
	3027	13.0	41.10	10.02	32.1
	3041	11.0	23.49	6.46	23.3
	3042	11.2	19.33	11.15	44.0
	3043	10.4	18.02	9.84	33.0
	3044	10.2	17.89	6.16	23.4
	3045	11.6	22.28	7.17	28.2
	3046	14.5	53.77	5.57	19.7
	3047	15.3	76.25	6.62	23.9
	3048	20.0	157.57	5.99	22.0
	3049	15.9	89.55	6.90	25.9
	3050	13.0	38.39	7.38	26.0
	3051	21.3	164.94	11.10	22.0
	3052	15.6	76.42	5.96	41.1
21:00 h	306	12.8	34.24	7.10	21.8
	307	13.5	41.27	8.77	24.9
	308	13.6	42.03	7.56	23.0
	309	11.8	25.86	10.54	28.5
	3010	10.4	23.32	7.89	25.6
	3028	12.1	30.78	4.28	23.3
	3029	12.0	26.65	5.63	19.5
	3030	10.8	23.29	11.83	47.1
	3031	11.6	26.40	6.53	22.2
	3053	12.3	39.31	4.91	19.6
	3054	11.5	31.97	7.69	26.7
	3055	13.6	48.16	6.77	24.7
	3056	12.1	34.06	6.44	25.1
	3057	14.4	55.36	6.45	22.5
	3058	13.7	50.74	7.57	28.7

APPENDIX

TABLE 13 : Length, weight, hemoglobin content and packed cell volume raw data for goldfish acclimated to a cycling temperature of $25^{o} \pm 5^{o}C$.

SAMPLING TIME	CODE NO.	FORK LENGTH (cm)	WEIGHT (gm)	Hb CONTENT (gm/100ml)	PCV (%)
03:00 h	cy 11	13.5	37.83	7.39	20.7
	cy 12	13.5	44.85	8.58	27.7
	cy 13	12.4	31.14	7.64	27.5
	cy 14	14.4	47.44	7.87	2.69
	cy 15	12.2	33.86	7.72	25.6
	cy 36	11.1	26.52	6.72	22.6
	cy 37	11.2	28.06	8.03	29.4
	cy 38	11.2	28.56	7.71	26.9
	cy 39	12.2	37.25	8.85	30.4
	cy 40	12.0	31.88	10.58	36.5
	cy 59	11.1	30.50	8.22	30.8
	cy 60	11.4	32.37	8.55	33.2
	cy 61	10.5	22.01	8.35	30.8
	cy 62	11.8	30.82	9.20	33.9
	cy 63	11.6	30.96	7.47	30.1
	cy 67	10.8	23.47	8.96	29.7
	cy 68	10.5	20.87	8.35	26.5
09:00 h	cy 16	15.0	50.98	9.14	29.1
	cy 17	14.1	46.11	8.88	28.3
	cy 18	13.9	39.74	8.39	28.7
	cy 19	12.0	27.06	8.91	31.3
	cy 20	11.8	26.06	8.54	25.5
	cy 41	12.2	37.63	9.80	33.3
	cy 42	10.2	21.21	9.41	32.5
	cy 43	11.5	29.66	8.35	28.3
	cy 44	11.4	27.61	9.13	29.7
	cy 45	11.0	24.51	10.23	34.8
	cy 54	11.4	29.52	6.03	23.4
	cy 55	11.3	27.87	6.10	22.0
	cy 56	10.3	20.93	7.31	28.4
	cy 57	11.9	42.26	7.91	29.1
	cy 58	11.1	29.56	7.91	29.5
	cy 69	10.5	28.78	8.10	26.9
	cy 70	11.1	24.98	8.74	29.9
	cy 71	10.3	21.69	10.19	35.1
15:00 h	cy 1	11.9	27.42	8.99	33.9
	cy 2	15.4	77.62	7.12	23.4
	cy 3	13.9	39.51	8.17	28.1
	cy 4	10.8	18.63	7.42	29.9
	cy 5	12.3	33.20	7.49	26.7
	cy 21	12.4	24.67	8.08	30.6
	cy 22	12.9	32.59	7.56	30.2
	cy 23	12.9	35.66	7.13	27.0
	cy 24	13.0	36.05	5.92	25.0
	cy 25	12.8	32.39	8.30	29.0
	cy 26	13.1	43.78	8.33	30.6
	cy 27	12.1	33.25	8.61	32.0
	cy 28	10.3	21.25	8.09	29.2
	cy 29	12.3	36.62	8.25	31.0
	cy 30	12.6	46.60	9.98	38.2
	cy 46	12.2	25.16	9.34	33.4
	cy 47	11.0	25.73	7.97	27.0
	cy 48	12.2	37.12	9.52	35.3
21:00 h	cy 6	12.1	26.89	7.79	29.0
	cy 7	12.2	34.05	7.27	25.2
	cy 8	12.0	30.72	10.26	35.7
	cy 9	14.0	48.73	8.20	24.8
	cy 10	13.9	44.80	5.26	21.9
	cy 31	12.1	38.93	9.02	29.4
	cy 32	11.5	29.06	8.57	31.0
	cy 33	11.6	27.06	9.62	31.5
	cy 34	11.2	26.05	7.33	27.9
	cy 35	11.6	29.92	8.49	31.9
	cy 49	12.1	42.73	9.43	33.5
	cy 50	10.8	23.39	7.27	27.9
	cy 51	11.7	27.74	8.55	29.9
	cy 52	11.8	31.06	7.55	26.9
	cy 53	10.7	26.64	8.01	30.9
	cy 64	11.1	25.48	7.77	24.7
	cy 65	10.9	23.18	8.90	29.0
	cy 66	11.1	26.81	9.13	28.8

APPENDIX

TABLE 14 : Plasma water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 20°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT
		(mmol/l)							(kg/l)
03:00 h	203	132.29	2.98	0.73	2.08	138.08	114.75	252.83	0.9570
	204	133.71	2.88	0.61	1.71	138.91	108.20	247.11	0.9565
	2015	-	-	-	-	-	115.10	-	-
	2016	131.16	4.89	0.59	1.88	138.52	119.57	258.09	0.9457
	2021	135.47	3.29	0.90	2.04	141.70	106.50	248.20	0.9575
	2022	137.05	3.34	1.00	2.32	143.71	109.68	253.39	0.9792
	2023	139.42	3.18	1.04	2.10	145.74	113.49	259.23	0.9579
	2024	139.42	2.60	0.98	2.10	145.10	109.99	255.09	0.9681
	2025	141.00	3.32	0.99	2.69	148.00	113.17	261.17	0.9684
	2026	131.53	2.37	1.11	2.78	137.79	108.40	246.19	0.9570
	2027	135.47	2.16	1.00	2.69	141.32	109.99	251.31	0.9688
	2028	129.95	2.82	0.91	2.60	136.28	103.00	239.28	0.9579
	2029	133.89	3.47	1.04	2.87	141.27	109.04	250.31	0.9588
	2030	135.47	2.65	1.03	2.41	141.56	114.44	256.00	0.9677
	2031	128.59	1.70	0.99	2.64	133.92	106.84	240.76	0.9681
	2032	126.85	2.32	0.82	2.58	132.57	101.40	233.97	0.9688
	2033	125.99	2.16	0.74	2.42	131.32	100.76	232.08	0.9579
	2034	107.77	2.14	0.61	1.37	111.89	91.48	203.37	0.9792
	2035	129.46	2.39	0.79	5.29	137.93	98.80	236.73	0.9579
09:00 h	205	133.15	3.34	0.55	2.10	139.14	111.99	251.13	0.9070
	206	-	-	-	-	-	-	-	-
	2017	133.15	3.28	0.84	2.13	139.40	125.43	264.83	0.9479
	2018	132.29	3.02	0.58	1.86	137.75	119.92	257.67	0.9247
	2036	137.26	2.60	0.96	2.34	143.16	110.36	253.52	0.9579
	2037	137.26	2.73	0.93	2.34	143.26	107.80	251.06	0.9579
	2038	137.26	3.18	0.88	2.46	143.78	115.16	258.94	0.9479
	2039	137.26	2.98	0.94	2.19	143.37	111.00	254.37	0.9688
	2040	126.85	2.24	0.90	1.89	131.88	105.24	127.12	0.9688
	2041	135.52	2.91	0.96	2.21	141.60	109.12	250.72	0.9579
	2047	134.64	2.56	0.95	2.28	140.43	106.54	246.97	0.9474
	2048	134.64	3.22	1.01	2.21	141.08	110.08	251.16	0.9479
	2049	135.52	2.43	0.93	2.24	141.12	107.51	248.63	0.9381
	2050	139.04	3.16	1.01	2.68	145.89	114.59	260.48	0.9381
	2051	128.20	2.41	0.95	2.22	133.78	110.08	243.86	0.9375
	2052	133.39	2.65	0.94	2.16	139.14	112.66	251.80	0.9579
	2070	139.20	3.03	0.81	2.38	145.42	109.68	255.10	0.9368
	2071	142.68	3.01	0.86	2.51	149.06	111.58	260.64	0.9271
15:00 h	207	137.40	2.79	0.73	1.91	142.83	117.16	259.99	0.9263
	208	132.29	2.92	0.68	1.78	137.67	116.47	254.14	0.9579
	2011	138.54	3.42	0.84	1.67	144.47	113.72	258.19	0.9474
	2012	139.11	3.18	0.70	1.97	144.96	117.51	262.47	0.9684
	2019	130.88	2.53	0.74	1.76	135.91	123.02	258.93	0.9326
	2020	133.43	2.82	0.92	1.83	139.00	118.20	257.20	0.9579
	2053	144.64	2.75	1.07	2.55	151.01	105.58	256.59	0.9479
	2054	131.66	2.93	1.20	4.01	139.80	103.32	243.12	0.9375
	2055	134.25	2.41	1.02	2.28	139.96	112.98	252.94	0.9388
	2056	132.52	3.20	1.00	2.31	139.03	110.08	249.11	0.9362
	2057	132.52	3.09	1.10	2.22	138.93	111.69	250.62	0.9681
	2063	136.59	2.98	1.02	2.51	143.10	109.68	252.78	0.9579
	2064	142.68	3.03	0.97	2.54	149.22	107.45	256.67	0.9158
	2065	135.72	2.65	0.92	2.54	141.83	111.90	253.73	0.9271
	2066	134.85	2.73	1.16	3.63	142.37	103.22	245.69	0.9278
	2067	131.37	3.74	1.04	2.76	138.91	103.00	241.91	0.9368
	2072	140.07	2.80	0.91	2.32	146.10	112.86	258.96	0.9468
	2073	134.85	3.58	0.95	2.85	142.23	112.54	254.77	0.9263
21:00 h	201	133.43	2.86	0.72	1.86	138.87	116.47	255.34	0.9574
	202	133.71	3.04	0.63	2.18	139.56	106.82	246.38	0.9355
	209	136.84	3.12	1.10	2.25	143.31	-	-	0.9412
	2010	132.01	3.38	0.70	1.83	137.92	112.34	250.26	0.9474
	2013	130.02	3.88	0.64	2.08	136.62	106.14	242.76	0.9892
	2014	134.85	3.22	0.75	2.06	140.88	109.58	250.46	0.9355
	2042	135.52	3.03	1.08	2.95	142.58	110.73	253.31	0.9479
	2043	132.88	3.13	0.97	2.21	139.19	102.68	241.87	0.9579
	2044	135.52	3.00	1.08	2.55	142.15	106.86	249.01	0.9583
	2045	136.40	2.82	0.97	2.49	142.68	112.98	255.66	0.9368
	2046	139.04	3.24	0.97	2.52	145.77	115.23	261.00	0.9468
	2058	125.60	3.23	0.90	2.28	132.01	114.91	246.92	0.9474
	2059	128.20	3.64	1.08	2.55	135.47	107.18	242.65	0.9479
	2060	141.17	3.75	1.00	2.58	148.50	108.47	256.97	0.9449
	2061	145.29	2.78	0.99	2.57	151.63	112.66	264.29	0.9381
	2062	138.33	3.87	1.02	2.60	145.82	110.40	256.22	0.9263
	2068	141.81	3.11	0.99	2.67	148.58	108.72	257.30	0.9375
	2069	140.07	3.25	0.86	2.35	146.53	109.68	256.21	0.9468

APPENDIX

TABLE 15 : Plasma water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 25°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/l)
		(mmol/l)							
03:00 h	254	120.66	2.60	0.46	2.06	125.78	117.13	242.91	0.9355
	255	116.12	2.77	0.52	1.87	121.28	113.69	234.97	0.9574
	266	127.19	2.71	0.71	2.26	132.87	120.34	253.21	0.9574
	2522	130.59	2.95	0.55	1.77	135.86	107.83	243.69	0.9674
	2523	131.73	3.28	0.57	2.00	137.58	113.69	251.27	0.9574
	2524	51.131	1.48	0.29	2.27	55.17	119.20	174.37	-
	2535	127.98	2.68	0.68	5.63	136.97	106.69	243.66	0.9293
	2536	132.52	3.01	-	-	-	114.41	-	0.9462
	2537	120.60	2.98	0.68	1.88	126.14	102.33	228.47	0.9271
	2564	128.71	2.69	0.74	2.08	134.22	111.19	245.41	0.9171
	2565	129.26	2.25	0.83	2.06	134.40	114.35	248.75	0.9255
	2566	135.79	2.38	0.74	2.46	141.37	113.09	254.46	0.9579
	2567	134.70	2.66	0.78	2.13	140.27	114.67	254.94	0.9263
	2568	135.25	2.47	0.88	2.31	140.91	109.92	25.083	0.9355
	2569	133.62	2.09	0.67	2.42	138.80	105.49	244.29	0.9341
	2570	134.70	1.89	0.74	2.48	139.81	105.17	244.98	0.9457
	2571	131.98	2.38	0.85	2.44	137.65	105.80	243.45	0.9255
09:00 h	257	135.13	2.54	0.52	2.53	140.72	120.58	261.30	0.9787
	258	57.37	1.56	0.29	3.19	62.41	-	-	0.9130
	259	126.90	2.93	0.86	2.57	133.26	108.52	241.78	0.9667
	2525	127.75	3.71	0.51	2.28	134.25	114.38	248.63	0.9570
	2526	48.01	1.83	0.11	3.04	52.99	109.55	162.54	0.9833
	2527	128.89	3.32	0.51	2.31	135.03	115.41	250.44	0.9681
	2538	131.95	3.22	0.69	1.45	137.31	113.74	251.05	0.9485
	2539	131.39	3.45	0.31	1.64	136.79	115.08	251.87	0.9674
	2540	137.06	3.20	0.79	2.51	143.56	117.43	260.99	0.9462
	2544	138.64	2.44	0.92	2.20	144.20	112.39	256.59	0.9574
	2545	141.89	2.67	0.93	2.42	147.91	115.54	263.45	0.9479
	2546	138.10	2.46	0.93	2.25	143.74	113.33	257.07	0.9451
	2547	138.10	2.49	0.92	2.05	143.56	112.07	255.63	0.9565
	2548	138.64	2.83	0.81	2.03	144.31	112.07	256.38	0.9681
	2549	139.73	3.07	0.88	2.18	145.86	110.81	256.67	0.9574
	2550	138.64	3.21	0.67	2.14	144.66	110.19	254.85	0.9462
	2551	138.10	2.97	0.81	2.29	144.17	109.87	254.04	0.9355
	2552	142.43	2.91	0.90	2.03	148.27	110.19	258.46	0.9570
	2553	140.27	2.94	0.66	2.10	145.97	110.50	256.47	0.9780
15:00 h	2510	135.70	4.08	0.68	2.88	143.34	120.58	263.92	-
	2511	128.61	3.63	0.57	2.28	135.09	116.10	251.19	0.9468
	2512	129.46	3.44	0.57	2.24	135.71	114.38	250.09	0.8421
	2516	128.04	3.18	0.59	2.04	133.85	109.21	243.06	0.8462
	2517	129.74	2.85	0.82	2.24	135.65	115.06	250.71	0.9681
	2518	-	-	-	-	-	-	-	-
	2528	128.61	4.73	0.57	2.31	136.22	112.31	248.53	0.9362
	2529	129.46	4.08	0.71	2.52	136.77	107.83	244.60	0.9429
	2530	138.77	4.36	0.63	-	-	113.40	-	0.8355
	2531	138.48	3.23	0.92	1.64	144.27	106.02	250.29	0.9375
	2541	140.18	2.60	0.82	2.72	146.32	116.08	262.40	0.9255
	2542	134.79	3.14	0.58	1.80	140.31	117.43	257.74	0.9485
	2543	134.79	3.94	0.76	3.10	142.59	114.74	257.33	0.9375
	2554	126.76	1.88	1.02	2.56	132.22	112.77	244.99	0.9348
	2555	132.32	2.39	1.06	2.62	138.39	113.72	252.11	0.9468
	2556	128.99	2.95	0.99	2.31	135.24	111.51	246.75	0.9348
	2557	130.10	2.37	1.01	2.45	135.93	108.66	244.59	0.9043
	2558	130.65	2.21	1.02	2.60	136.48	115.30	251.78	0.9362
21:00 h	251	131.73	3.10	0.51	2.20	137.54	116.10	253.64	0.9368
	252	-	-	-	-	-	-	-	-
	253	118.96	3.12	0.49	1.85	124.42	108.86	233.28	0.9286
	2513	130.02	2.83	0.56	1.81	135.22	115.75	250.97	0.9565
	2514	124.06	2.77	0.55	1.87	129.25	118.86	248.11	0.9555
	2515	125.48	3.03	0.58	2.06	131.15	114.72	245.87	0.9677
	2519	133.15	4.30	0.60	2.06	140.11	113.69	253.80	0.9792
	2520	132.29	5.57	0.69	2.47	141.02	110.93	251.95	0.9583
	2521	128.61	4.32	0.62	1.87	135.42	108.17	243.59	0.9512
	2532	137.35	3.15	0.64	2.61	143.75	109.04	252.79	0.9670
	2533	134.23	3.33	0.95	1.50	140.01	112.73	252.74	0.9583
	2534	132.90	3.52	0.90	1.50	138.82	113.74	252.56	0.9670
	2559	133.99	2.70	0.77	2.08	139.54	105.49	245.03	0.9570
	2560	140.10	2.04	0.92	2.25	145.31	115.94	261.25	0.9570
	2561	142.33	2.67	0.92	2.29	148.21	114.04	262.25	0.9355
	2562	140.10	2.47	0.93	2.34	145.84	110.24	256.08	0.9355
	2563	136.77	2.87	0.93	2.27	142.84	113.09	255.93	0.9457

APPENDIX

TABLE 16 : Plasma water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 30°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/l)
		(mmol/l)							
03:00 h	3011	127.23	2.73	0.85	2.40	133.21	113.69	246.90	0.9375
	3012	121.21	3.73	0.98	2.82	128.74	98.19	226.93	0.9457
	3013	128.95	3.07	0.86	2.71	135.59	111.28	246.87	0.9783
	3014	125.51	2.51	0.94	2.54	131.50	117.13	248.63	0.9362
	3015	131.54	2.73	1.00	3.11	138.38	113.69	252.07	0.9677
	3032	132.68	3.08	1.14	2.07	138.97	110.24	249.21	0.9677
	3033	134.41	1.80	1.03	2.84	140.08	107.14	247.22	0.9451
	3034	130.68	2.33	1.03	1.80	135.84	110.24	246.08	0.9560
	3035	137.28	2.47	1.02	2.51	143.28	108.86	252.14	0.9574
	3059	140.29	2.74	1.04	2.65	146.72	109.91	256.63	0.9375
	3060	137.11	2.95	1.04	2.65	143.75	114.60	258.35	0.9368
	3061	137.11	2.85	1.04	2.39	143.39	117.93	261.32	0.9271
	3062	136.31	2.85	0.92	2.32	142.40	114.91	257.31	0.9381
	3063	135.51	3.47	0.98	2.22	142.18	111.88	254.06	0.9255
	3064	134.72	3.26	0.95	2.43	141.36	117.93	259.29	0.9167
09:00 h	3016	131.25	2.85	0.98	2.62	137.70	117.82	255.52	0.9570
	3017	135.84	2.51	1.12	2.48	141.95	114.03	255.98	0.9462
	3018	130.68	3.10	1.23	3.45	138.46	118.51	256.97	0.9574
	3019	134.41	3.45	1.13	3.30	142.29	115.07	257.36	0.9570
	3036	129.53	3.11	1.04	2.32	136.00	107.83	243.83	0.9468
	3037	134.12	2.61	0.82	1.84	139.39	109.90	249.29	0.9560
	3038	133.55	2.27	1.18	2.25	139.25	111.28	250.53	0.9368
	3039	131.25	2.94	1.09	2.41	137.69	108.86	246.55	0.9579
	3040	143.02	2.89	1.14	2.57	149.62	108.69	258.31	0.9579
	3065	138.70	2.74	0.95	2.34	144.73	119.61	264.34	0.9375
	3066	137.11	2.83	0.98	2.36	143.28	119.61	262.89	0.9167
	3067	137.90	2.74	0.95	2.32	143.91	121.28	265.19	0.9381
	3068	137.11	2.74	1.02	2.29	143.16	118.93	262.09	0.9278
	3069	134.72	2.88	0.93	2.22	140.75	120.61	261.36	0.9271
	3070	135.51	3.43	0.96	2.41	142.31	117.26	259.57	0.9271
15:00 h	301	125.94	3.13	1.02	3.02	133.11	120.92	254.03	0.9565
	302	128.67	2.70	1.13	3.19	135.69	113.00	248.69	0.9681
	303	128.38	3.38	1.06	2.99	135.81	118.86	254.67	0.9789
	304	128.67	2.23	0.85	2.08	133.83	116.79	250.62	0.9468
	305	130.68	2.51	0.96	2.25	136.40	115.06	251.46	0.9583
	3020	130.10	3.73	1.06	2.57	137.46	111.97	249.43	0.9457
	3021	130.68	2.78	1.05	2.31	136.82	115.41	252.23	0.9462
	3022	131.25	1.18	0.89	2.28	135.60	113.00	248.60	0.9355
	3023	128.95	2.83	0.85	2.79	135.42	117.48	252.90	0.9457
	3024	128.09	2.38	0.99	3.13	134.59	114.72	249.31	0.9474
	3025	120.06	2.22	0.68	2.02	124.98	90.26	215.24	0.9468
	3026	153.35	3.78	1.37	3.50	162.00	105.42	267.42	0.9588
	3027	139.29	3.11	1.23	3.39	147.02	113.69	260.71	0.9670
	3041	130.10	2.64	1.16	2.54	136.44	119.20	255.64	0.9565
	3042	126.94	2.13	0.72	2.03	131.82	114.37	246.19	0.9574
	3043	128.67	3.20	0.82	2.54	135.23	110.59	245.82	0.9570
	3044	127.81	2.52	1.01	2.67	134.01	109.90	243.91	0.9468
	3045	130.10	2.58	0.87	2.19	135.74	112.31	248.05	0.9681
	3046	136.73	2.54	0.92	2.28	142.47	115.67	258.14	0.9588
	3047	132.67	2.97	0.94	2.16	138.74	113.34	252.08	0.9575
	3048	133.48	3.44	0.97	1.94	139.83	115.67	255.50	0.9783
	3049	132.67	3.64	0.98	2.13	139.42	120.33	259.75	0.9681
	3050	131.86	3.29	0.94	2.20	138.29	117.00	255.29	0.9570
	3051	139.97	3.77	1.14	2.35	147.23	115.67	262.90	0.9479
	3052	133.48	3.88	0.92	1.72	140.00	113.24	253.24	0.9790
21:00 h	306	130.96	2.26	1.05	2.82	137.09	114.72	251.81	0.9479
	307	126.94	2.20	0.92	2.37	132.43	108.52	240.95	0.9579
	308	144.45	1.98	1.19	3.64	151.26	108.86	260.12	0.9457
	309	131.82	2.98	1.26	4.24	140.30	115.75	256.05	0.9691
	3010	125.80	2.76	0.91	2.54	132.01	116.45	248.46	0.9789
	3028	131.25	2.89	1.10	2.29	137.53	108.17	245.70	0.9574
	3029	132.68	3.36	0.99	2.26	139.29	115.41	254.70	0.9368
	3030	137.56	2.97	1.18	2.04	143.75	110.24	253.99	0.9574
	3031	134.98	2.83	1.32	4.80	143.93	109.55	253.48	0.9468
	3053	131.86	3.07	0.94	2.42	138.29	112.67	250.96	0.9583
	3054	131.86	2.63	0.88	2.54	137.91	107.33	245.24	0.9583
	3055	135.91	2.64	0.87	2.16	141.58	118.34	259.92	0.9579
	3056	131.86	2.74	0.93	2.30	137.83	114.93	252.76	0.9479
	3057	131.05	3.22	0.97	2.16	137.40	112.92	250.32	0.9479
	3058	133.48	3.14	1.02	2.32	139.96	114.93	254.89	0.9381

APPENDIX

TABLE 17 : Plasma water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to a cycling temperature of $25^o \pm 5^oC$.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/l)
		(mmol/l)							
03:00 h	cy 11	116.89	2.56	0.68	2.43	122.56	81.41	203.97	0.9462
	cy 12	127.35	2.62	1.04	2.21	133.22	103.64	236.86	0.9468
	cy 13	115.62	2.60	1.05	2.05	121.32	90.56	211.88	0.9468
	cy 14	111.81	1.82	0.52	2.07	116.22	87.95	204.17	0.9574
	cy 15	118.16	2.52	1.20	2.29	124.17	79.45	203.62	0.9574
	cy 36	131.35	2.75	0.72	2.22	137.04	106.89	243.93	0.9583
	cy 37	132.92	2.92	0.69	2.19	138.72	106.89	245.61	0.9479
	cy 38	131.46	2.80	0.95	2.34	137.55	109.80	247.35	0.9263
	cy 39	130.57	3.00	0.92	2.55	137.04	106.56	243.60	0.9158
	cy 40	133.23	2.92	0.99	3.08	140.22	109.48	249.70	0.9255
	cy 59	133.50	2.65	0.91	2.38	139.44	108.51	247.95	0.9479
	cy 60	130.90	2.83	0.85	2.22	136.80	103.65	240.45	0.9381
	cy 61	131.60	2.32	0.88	2.38	137.18	107.21	244.39	0.9381
	cy 62	122.71	2.85	0.84	2.31	128.71	106.89	235.60	0.9271
	cy 63	130.71	2.82	0.78	2.28	136.59	104.94	241.53	0.974
	cy 67	131.60	2.17	0.95	2.05	136.77	98.17	234.94	0.9362
	cy 68	130.71	2.56	0.74	2.28	136.29	100.43	236.72	0.9681
09:00 h	cy 16	110.63	2.44	0.93	2.29	116.29	97.95	214.04	0.9579
	cy 17	120.80	2 79	1.08	2.34	127.01	91.87	218.88	0.9341
	cy 18	109.04	2.38	0.66	2.12	114.20	85.33	199.53	0.9457
	cy 19	109.99	2.72	1.32	2.40	116.43	79.77	196.20	0.9457
	cy 20	126.84	3.18	1.35	2.38	133.75	100.04	233.79	0.9677
	cy 41	127.01	2.65	0.91	2.34	132.91	103.97	236.88	0.9167
	cy 42	133.23	2.93	0.88	2.39	139.43	111.42	250.85	0.9278
	cy 43	122.57	2.68	0.79	2.02	128.06	106.24	234.30	0.9375
	cy 44	131.46	2.82	0.87	2.78	137.93	111.10	249.03	0.9271
	cy 45	133.23	3.54	0.88	2.76	140.41	108.51	248.92	0.9375
	cy 54	142.15	2.68	0.69	2.45	147.97	107.16	255.13	0.9479
	cy 55	140.42	2.78	0.84	2.36	146.40	105.24	251.64	0.9479
	cy 56	136.96	3.18	0.68	2.10	142.92	105.92	248.84	0.9381
	cy 57	135.23	2.75	0.91	2.33	141.22	108.83	250.05	0.9368
	cy 58	136.96	3.15	0.67	2.38	143.16	109.48	252.64	0.9375
	cy 69	131.60	2.56	0.67	2.43	137.26	99.78	237.04	0.9368
	cy 70	131.60	2.71	0.75	2.28	137.34	106.22	243.56	0.9375
	cy 71	124.49	3.15	0.61	2.28	130.53	100.43	230.96	0.9362
15:00 h	cy 1	119.66	2.97	0.48	2.72	125.83	78.14	203.97	0.9565
	cy 2	133.87	2.37	0.75	2.43	139.42	110.83	250.25	0.9574
	cy 3	128.65	1.93	0.64	2.76	133.98	100.04	234.02	0.9438
	cy 4	117.63	2.39	0.69	3.02	123.73	88.27	212.00	0.9462
	cy 5	137.35	3.23	0.93	2.84	144.35	104.62	248.97	0.9444
	cy 21	103.95	2.46	0.65	2.54	109.60	84.35	193.95	0.9239
	cy 22	120.48	2.52	1.37	2.34	126.71	88.27	214.98	0.9348
	cy 23	117.94	2.99	0.90	2.10	123.93	104.95	228.88	0.9560
	cy 24	126.52	2.60	1.16	2.32	132.60	104.62	237.22	0.9362
	cy 25	123.35	2.75	1.18	2.78	130.06	98.73	228.79	0.9355
	cy 26	141.58	3.04	0.97	3.43	149.02	109.56	258.58	0.9375
	cy 27	137.64	2.66	0.95	2.50	143.75	111.20	254.95	0.9479
	cy 28	95.14	3.85	0.68	2.33	102.00	106.94	208.94	0.9479
	cy 29	136.86	3.22	0.85	3.00	143.93	110.22	254.15	0.9375
	cy 30	137.64	3.20	0.91	2.98	144.73	109.89	254.62	0.9362
	cy 46	130.57	2.82	0.74	2.34	136.47	106.84	243.31	0.9474
	cy 47	131.46	2.93	0.80	2.83	138.02	106.20	244.22	0.9479
	cy 48	132.34	3.33	0.86	2.57	139.10	109.40	248.50	0.9479
21:00 h	cy 6	120.24	2.90	1.04	2.31	126.49	83.04	209.53	0.9457
	cy 7	134.16	2.77	0.99	2.23	140.15	105.60	245.75	0.9583
	cy 8	126.04	2.66	1.24	2.29	132.23	77.48	209.71	0.9355
	cy 9	132.13	2.26	0.92	2.05	137.36	100.04	237.40	0.9362
	cy 10	143.15	2.64	0.93	2.25	148.97	106.91	255.88	0.9457
	cy 31	133.71	2.45	0.99	3.02	140.17	103.98	244.15	0.9375
	cy 32	132.92	2.79	0.74	2.10	138.55	109.23	247.78	0.9583
	cy 33	129.77	3.02	0.88	2.31	135.98	107.26	243.24	0.9579
	cy 34	132.92	2.94	0.95	3.17	139.98	107.92	247.90	0.9479
	cy 35	123.47	3.05	0.69	2.07	129.28	108.92	238.18	0.9263
	cy 49	127.01	1.99	0.87	2.32	132.19	105.88	238.07	0.9474
	cy 50	137.82	2.90	0.97	2.15	143.84	109.08	252.92	0.9381
	cy 51	139.55	3.00	1.11	4.17	147.83	107.48	255.31	0.9271
	cy 52	136.96	2.82	0.73	2.45	142.96	105.56	248.52	0.9368
	cy 53	136.96	3.18	0.66	2.33	143.13	106.84	249.97	0.9375
	cy 64	131.60	2.40	0.65	2.52	137.17	99.44	236.61	0.9474
	cy 65	125.38	2.41	0.84	2.33	130.96	95.55	226.51	0.9368
	cy 66	128.05	2.82	0.73	2.61	134.21	96.56	230.77	0.9368

APPENDIX

TABLE 18 : Erythrocytic water, electrolyte, total cation and total cation + Cl^- raw data for packed cells in goldfish acclimated to $20^{\circ}C$.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/kg)
		(mmol/l packed cells)							
03:00 h	203	16.64	106.02	9.21	0.261	132.13	64.05	196.18	0.6881
	204	12.61	90.05	7.26	0.807	110.73	57.43	168.16	0.7181
	2015	-	-	-	-	-	-	-	-
	2016	13.38	89.47	8.82	0.444	112.11	72.99	185.10	-
	2021	-	-	-	-	-	55.88	-	-
	2022	11.49	100.21	6.82	0.262	118.78	70.34	189.12	0.7147
	2023	11.42	104.14	8.16	0.249	123.97	66.49	190.46	0.7086
	2024	7.66	110.05	7.93	0.305	125.95	67.22	193.67	0.7271
	2025	16.47	102.17	7.61	0.270	126.52	66.88	193.40	0.6394
	2026	6.11	111.53	8.48	0.315	126.44	68.51	194.95	0.6925
	2027	11.54	106.13	7.77	0.251	125.69	71.45	197.14	0.6242
	2028	5.20	91.78	6.45	0.603	104.03	86.58	190.61	0.6286
	2029	9.31	101.68	7.59	0.683	119.26	80.81	200.07	0.7206
	2030	7.99	106.12	7.69	0.316	122.12	71.32	193.44	0.6641
	2031	15.06	101.73	8.01	0.328	125.13	71.91	197.04	0.7194
	2032	16.44	101.71	8.09	0.330	126.57	-	-	-
	2033	17.31	96.76	8.10	0.518	122.69	73.21	195.90	0.7059
	2034	26.28	72.88	8.07	0.638	107.87	-	-	0.7364
	2035	14.59	99.75	8.32	0.185	122.85	55.73	178.58	0.6738
09:00 h	205	16.84	94.03	7.60	0.438	118.91	-	-	0.6834
	206	-	-	-	-	-	-	-	-
	2027	-	-	-	-	-	63.74	-	0.8024
	2018	11.13	94.48	7.86	0.535	114.01	72.23	186.24	0.6837
	2036	11.26	102.69	7.17	-	-	73.30	-	0.5284
	2037	7.39	107.59	7.48	0.307	122.77	68.53	191.30	0.7112
	2038	15.43	103.49	5.95	0.071	124.94	67.57	192.51	0.6204
	2039	15.16	106.43	6.56	0.320	128.47	-	-	-
	2040	13.73	102.61	6.98	0.266	123.59	69.72	193.31	0.6268
	2041	13.26	111.60	8.09	0.435	133.39	79.40	212.79	0.6738
	2047	14.59	101.55	9.08	0.299	125.52	75.63	201.15	0.7252
	2048	11.14	102.08	7.47	0.310	121.00	-	-	-
	2049	6.79	101.56	7.02	0.270	115.64	76.37	192.01	0.7186
	2050	12.52	105.88	8.21	0.288	126.90	74.25	201.15	0.7374
	2051	10.03	106.45	8.14	0.368	124.99	80.71	205.70	0.5650
	2052	8.15	104.81	8.22	0.312	121.49	59.29	180.78	0.6991
	2070	11.50	100.48	8.89	0.292	121.16	74.10	195.26	0.7092
	2071	11.18	106.01	9.50	0.279	126.97	72.57	199.54	0.6747
15:00 h	207	11.61	102.82	10.20	0.354	124.98	70.79	195.77	0.7240
	208	18.42	119.59	9.80	1.608	149.42	83.68	233.10	0.7000
	2011	12.03	91.23	6.07	0.718	110.05	66.35	176.40	0.6665
	2012	-	-	-	-	-	-	-	0.6808
	2019	-	-	-	-	-	-	-	0.6161
	2020	-	-	7.54	0.370	-	-	-	0.6633
	2053	8.05	99.37	6.16	0.339	113.92	82.68	196.60	0.7685
	2054	11.42	101.79	8.89	0.230	122.33	76.13	198.46	0.7564
	2055	9.63	104.47	6.61	0.271	120.98	83.21	204.19	0.7159
	2056	6.69	107.10	7.38	0.279	121.45	77.40	198.85	0.7256
	2057	8.19	103.91	7.30	0.273	119.67	77.72	197.39	0.7056
	2063	10.21	96.99	7.82	0.290	115.31	80.73	196.04	0.5335
	2064	8.38	104.90	8.20	0.332	121.81	-	-	-
	2065	14.19	105.47	8.28	0.278	128.22	87.65	215.87	0.7233
	2066	9.90	102.15	8.19	0.247	120.49	79.07	199.56	0.6962
	2067	11.94	103.78	8.73	0.281	124.73	86.07	210.80	0.5796
	2072	12.98	106.01	9.88	0.275	129.15	70.33	199.48	0.7253
	2073	14.43	108.20	9.57	0.278	132.48	-	-	-
21:00 h	201	14.17	95.64	6.85	0.445	117.11	-	-	0.6732
	202	-	-	-	-	-	65.79	-	-
	209	5.42	91.64	6.78	0.344	104.18	-	-	0.6819
	2010	7.33	88.83	6.98	0.268	103.41	69.42	172.83	0.7471
	2013	8.94	90.81	7.63	0.349	107.73	-	-	0.7246
	2014	8.14	93.63	7.60	0.350	109.72	77.07	186.79	0.7187
	2042	15.43	106.43	9.16	0.289	131.31	86.65	217.96	-
	2043	6.67	103.94	8.06	0.345	119.02	70.75	189.77	0.7344
	2044	10.89	103.17	6.71	0.320	121.09	74.47	195.56	0.6550
	2045	14.11	105.89	8.32	0.302	128.62	79.29	207.91	0.7516
	2046	11.44	102.08	7.63	0.282	121.43	73.84	195.27	0.6186
	2058	13.95	101.78	8.90	0.289	124.92	86.09	211.01	0.7252
	2059	10.45	97.51	7.53	0.298	115.79	79.69	195.48	0.8268
	2060	10.50	96.97	6.84	0.342	114.65	77.08	191.73	0.6656
	2061	12.31	97.00	7.83	0.315	117.46	72.91	190.37	0.7065
	2062	15.08	96.44	7.74	0.341	119.60	86.59	206.19	0.7109
	2068	13.36	103.24	10.18	0.292	127.07	67.14	194.21	0.7325
	2069	11.91	104.90	9.65	0.310	126.77	67.84	194.61	0,7022

APPENDIX

TABLE 19 : Erythrocytic water, electrolyte, total cation and total cation + Cl^- raw data for packed cells in goldfish acclimated to 25^oC.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/kg)
		(mmol/l packed cells)							
03:00 h	254	17.09	93.23	8.47	0.257	119.05	80.32	199.37	0.6779
	255	30.08	77.60	4.95	0.428	113.06	84.64	197.70	0.6925
	256	11.32	95.75	6.30	0.251	113.62	75.62	189.24	0.6898
	2522	6.20	98.68	7.96	0.348	113.19	81.66	194.85	0.6705
	2523	10.49	93.40	7.60	0.259	111.75	70.14	181.89	0.6551
	2524	-	-	-	-	-	-	-	-
	2435	21.24	91.74	6.09	-	-	72.75	-	0.6781
	2536	24.62	91.32	-	-	-	62.92	-	0.6523
	2537	15.00	92.57	9.22	-	-	62.24	-	0.6933
	2564	10.00	101.92	6.88	0.091	118.89	73.65	192.54	0.6840
	2565	9.88	99.54	7.95	0.224	117.59	49.30	166.89	0.6924
	2566	18.40	95.81	8.45	0.279	122.94	76.96	199.90	0.6296
	2567	-	-	-	-	-	77.28	-	0.6934
	2568	16.07	104.59	7.78	0.206	128.65	75.18	203.83	0.7398
	2569	18.47	99.01	8.53	0.197	126.21	58.14	184.35	0.7387
	2570	14.30	106.20	7.45	0.178	128.13	56.66	184.79	0.7939
	2571	15.61	104.86	8.03	0.230	128.73	48.80	177.53	0.7496
09:00 h	257	14.73	93.74	6.45	0.326	115.25	74.84	190.09	-
	258	-	-	-	-	-	-	-	-
	259	-	-	-	-	-	-	-	-
	2525	19.03	86.20	6.46	0.333	112.02	79.20	191.22	-
	2526	-	-	-	-	-	86.27	-	0.7410
	2527	8.63	91.96	2.76	0.250	103.60	76.33	179.93	-
	2538	20.96	94.42	6.81	0.134	122.32	64.31	186.63	0.6711
	2539	20.66	93.58	6.51	-	-	72.51	-	0.6932
	2540	-	-	-	0.190	-	64.54	-	0.6967
	2544	12.38	99.11	5.70	0.150	117.34	75.29	193.63	0.7319
	2545	16.32	89.55	7.67	0.109	113.65	82.34	195.99	0.6928
	2546	14.41	91.80	6.82	0.114	113.14	74.14	187.28	0.6742
	2547	13.06	88.43	7.07	0.032	108.59	72.67	181.26	0.6898
	2548	16.41	86.17	6.82	0.120	109.52	69.66	179.18	0.7109
	2549	16.60	78.86	6.90	0.133	102.49	81.73	184.22	0.6832
	2550	19.79	94.84	8.73	0.255	123.62	86.14	209.76	0.7277
	2551	23.26	92.93	10.28	0.084	126.55	84.63	211.18	0.7119
	2552	17.87	94.04	6.30	0.103	118.31	79.32	197.63	0.6583
	2553	18.61	90.10	7.00	0.101	115.81	80.83	196.64	0.6452
15:00 h	2510	9.14	89.16	5.46	0.150	103.91	82.52	186.43	-
	2511	17.07	98.25	7.78	0.333	123.43	-	-	-
	2512	9.32	88.67	7.54	-	-	75.79	-	-
	2516	12.76	95.79	6.94	-	-	-	-	-
	2517	13.14	95.32	7.74	0.418	116.62	82.97	199.59	0.6865
	2518	-	-	-	-	-	-	-	-
	2528	12.95	93.35	7.81	0.167	114.28	77.92	192.20	-
	2529	15.09	94.33	8.10	0.326	117.85	81.06	198.91	-
	2530	8.43	32.74	2.60	-	-	63.63	-	0.7187
	2531	7.22	94.22	6.29	-	-	64.19	-	0.6588
	2541	17.79	101.29	6.71	-	-	69.73	-	0.7109
	2542	20.96	96.71	7.21	-	-	78.62	-	0.7322
	2543	16.89	96.07	7.13	-	-	75.27	-	0.7118
	2554	8.24	92.38	5.70	0.070	106.39	81.90	188.29	0.6932
	2555	13.23	91.80	6.21	0.138	111.38	84.52	195.90	0.6680
	2556	12.66	85.61	6.39	0.095	104.76	72.09	176.85	0.6998
	2557	13.07	85.06	6.73	0.020	104.88	74.45	179.33	0.6783
	2558	5.96	105.13	6.04	0.076	117.21	79.55	196.76	0.6970
21:00 h	251	9.47	88.68	6.97	0.419	105.54	83.42	188.96	0.7422
	252	-	-	-	-	-	-	-	-
	253	21.64	91:20	6.97	0.263	120.07	82.86	202.93	0.7040
	2513	9.52	93.23	7.47	0.347	110.57	71.59	182.16	-
	2514	15.27	93.73	7.32	0.263	116.58	76.04	192.62	-
	2515	14.16	92.02	6.36	0.174	113.41	81.46	194.87	-
	2519	12.82	89.53	6.79	0.340	109.48	85.28	194.76	-
	2520	14.79	87.10	7.77	0.245	109.91	82.71	192.62	0.7086
	2521	8.42	85.70	7.24	0.263	101.62	-	-	-
	2532	22.69	93.38	5.93	-	-	77.83	-	0.6610
	2533	26.45	105.21	7.61	0.259	139.53	67.77	207.30	0.5057
	2534	18.40	93.17	6.23	-	-	68.77	-	0.6735
	2559	-	- -	-	-	-	-	-	-
	2560	7.21	105.67	6.79	0.086	119.76	76.88	194.64	0.6398
	2561	10.73	111.50	7.04	0.101	129.37	73.20	202.57	0.6557
	2562	8.33	106.72	7.70	0.116	122.87	77.79	200.66	0.6970
	2563	9.77	101.92	6.87	0.069	118.63	79.20	197.83	0.6712

APPENDIX

TABLE 20 : Erythrocytic water, electrolyte , total cation and total cation + Cl^- raw data for packed cells in goldfish acclimated to 30°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT
		(mmol/l packed cells)							(kg/kg)
03:00 h	3011	19.13	98.30	6.38	0.131	123.94	80.96	204.90	0.7065
	3012	9.24	93.97	7.74	0.066	111.02	71.88	182.90	0.6967
	3013	16.07	97.86	6.88	0.146	120.96	79.50	200.46	0.7243
	3014	15.56	98.86	5.89	0.101	120.41	73.23	193.64	0.6997
	3015	18.49	100.12	6.16	-	-	80.96	-	0.6580
	3032	19.[illegible]1	97.39	6.55	-	-	77.06	-	0.7533
	3033	27.36	103.79	7.52	0.026	138.70	83.68	222.38	0.7348
	3034	19.27	95.58	5.84	-	-	78.21	-	0.7512
	3035	21.02	99.09	6.40	0.084	126.59	72.88	199.47	-
	3059	18.13	107.45	6.63	0.070	132.28	71.30	203.58	0.6903
	3060	14.18	106.92	7.23	0.059	128.39	69.31	197.70	-
	3061	14.18	106.39	6.47	0.067	127.11	72.92	200.03	0.6747
	3062	13.25	107.98	7.54	0.116	128.89	73.01	201.90	0.6714
	3063	14.70	102.14	5.27	0.095	122.21	75.69	197.90	0.6782
	3064	9.50	105.85	6.02	0.100	121.47	80.33	201.80	0.6785
09:00 h	3016	-	-	-	-	-	79.69	-	0.7059
	3017	20.06	98.50	6.55	0.010	125.12	82.47	207.59	0.7443
	3018	16.68	99.79	6.95	0.124	123.54	83.87	207.41	0.7100
	3019	15.01	102.81	5.92	0.135	123.88	78.63	202.51	0.7086
	3036	25.32	89.15	6.82	0.234	121.52	87.26	208.78	0.7782
	3037	20.46	98.57	7.45	0.063	126.54	69.25	195.79	0.6583
	3038	22.21	97.54	6.08	-	-	77.98	-	0.6683
	3039	21.19	93.88	6.16	0.082	121.31	78.43	199.74	-
	3040	23.23	93.36	7.20	0.024	123.81	78.43	202.24	0.6742
	3065	13.18	105.87	6.70	0.092	125.84	78.06	203.90	0.6435
	3066	12.04	102.16	6.10	0.091	120.39	77.91	198.30	0.6750
	3067	12.26	107.98	5.72	0.092	125.05	82.03	208.08	0.6779
	3068	7.05	108.51	5.80	0.104	121.46	81.72	203.18	0.6406
	3069	11.16	105.33	7.01	0.095	123.60	81.67	205.27	0.6840
	3070	11.85	105.32	7.53	0.101	124.80	75.71	200.51	0.6622
15:00 h	301	11.75	98.82	5.89	0.061	116.52	84.69	201.21	0.6926
	302	31.52	146.73	7.71	0.819	186.78	91.88	278.66	0.7437
	303	21.87	103.78	6.61	0.139	132.40	77.01	209.41	0.7217
	304	17.17	102.16	7.01	-	-	71.27	-	0.7034
	305	14.56	101.61	6.53	-	-	76.73	-	0.7059
	3020	12.73	93.48	7.96	0.150	114.32	82.91	197.23	0.7323
	3021	21.28	97.40	5.92	0.073	124.67	82.05	206.72	0.7157
	3022	24.02	103.42	7.90	0.049	135.39	80.98	216.37	0.6809
	3023	14.53	89.16	7.07	0.067	110.83	78.56	189.39	0.7278
	3024	13.92	97.41	6.55	0.065	117.95	95.80	213.75	0.7252
	3025	15.01	82.40	8.97	0.319	106.70	72.49	179.19	0.7089
	3026	20.44	104.61	8.28	0.428	133.76	79.29	213.05	0.7630
	3027	9.13	104.99	7.18	0.050	121.35	75.24	196.59	0.7366
	3041	24.86	88.19	5.51	0.304	118.86	80.80	199.66	0.7086
	3042	18.29	93.39	6.97	0.032	118.68	71.79	190.47	0.6832
	3043	20.05	90.11	7.41	0.304	117.87	76.85	194.72	0.6803
	3044	21.40	93.04	7.07	0.529	122.04	79.72	201.96	0.7089
	3045	25.33	97.01	7.37	0.053	129.76	79.85	209.61	-
	3046	18.87	100.13	6.08	0.069	125.15	74.62	199.77	0.7085
	3047	12.72	103.52	5.79	0.030	122.06	80.74	202.80	0.7180
	3048	16.27	101.28	5.39	0.012	122.95	79.92	202.87	0.6985
	3049	12.24	102.39	5.78	0.031	120.44	85.46	205.90	0.6800
	3050	19.90	98.51	6.57	0.040	125.02	80.64	205.66	0.6809
	3051	27.29	90.71	5.70	0.061	123.76	80.30	204.06	0.6680
	3052	12.46	102.38	6.65	0.080	121.57	68.64	190.21	0.6702
21:00 h	306	17.11	99.96	6.69	0.067	123.83	86.41	210.24	0.7466
	307	10.41	98.87	5.82	0.056	115.16	79.63	194.79	0.7031
	308	22.25	104.92	7.56	0.019	134.75	85.42	220.17	0.7395
	309	22.83	107.09	7.16	0.263	137.34	74.00	211.34	0.6799
	3010	15.78	97.75	6.61	0.067	120.41	79.01	199.42	0.7106
	3028	25.49	93.51	8.32	0.311	127.63	75.40	203.03	0.8185
	3029	22.58	99.78	8.29	0.159	130.81	-	-	-
	3030	17.89	94.14	6.31	0.097	118.44	79.91	198.35	0.7181
	3031	24.34	102.82	8.05	0.248	135.46	-	-	0.7347
	3053	12.74	101.29	6.49	0.059	120.58	91.12	212.70	0.6831
	3054	15.60	94.63	6.49	0.068	116.79	69.56	186.35	-
	3055	11.19	102.42	7.35	0.079	121.04	71.89	191.93	0.6381
	3056	10.59	101.86	6.65	0.063	119.16	72.27	191.43	0.6647
	3057	11.81	95.17	6.18	0.067	113.23	82.70	195.93	0.8264
	3058	13.17	100.18	6.65	0.062	120.06	64.48	184.54	0.6837

APPENDIX

TABLE 21 : Erythrocytic water, electrolyte, total cation and total cation + Cl^- raw data for packed cells in goldfish acclimated to a cycling temperature of 25°± 5°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/kg)
		(mmol/l packed cells)							
03:00 h	cy 11	9.09	98.69	6.79	0.059	114.63	68.30	182.93	0.7323
	cy 12	-	103.54	5.86	0.061	-	72.91	-	0.7227
	cy 13	10.51	99.22	7.07	0.146	116.95	68.78	185.73	0.7298
	cy 14	5.31	98.71	6.74	0.110	110.87	63.22	174.09	0.7100
	cy 15	7.85	101.02	8.36	0.076	117.31	60.08	177.39	-
	cy 36	9.83	97.52	7.80	0.069	115.22	-	-	0.6644
	cy 37	6.65	105.93	7.50	0.105	120.19	76.77	196.96	0.6531
	cy 38	8.27	109.93	7.49	0.105	124.90	75.54	200.44	0.6717
	cy 39	8.98	107.99	8.59	0.095	125.66	69.87	195.53	0.6910
	cy 40	8.67	108.51	8.51	0.079	125.77	73.24	199.01	0.6944
	cy 59	5.36	102.66	5.80	0.101	113.92	76.15	190.07	0.6741
	cy 60	5.87	106.47	5.97	0.110	118.42	74.74	193.16	0.6714
	cy 61	-	-	-	-	-	74.64	-	0.6940
	cy 62	7.76	105.07	7.73	0.118	120.68	68.85	189.53	0.6717
	cy 63	8.19	107.71	7.91	0.119	123.93	69.68	193.61	0.6928
	cy 67	14.07	105.09	8.16	0.149	127.47	67.81	195.28	0.6836
	cy 68	9.72	103.50	7.74	0.179	121.14	74.81	195.95	-
09:00 h	cy 16	6.93	102.62	5.78	0.075	115.41	74.96	190.37	0.7488
	cy 17	11.20	104.75	7.84	0.078	123.87	69.12	192.99	0.7038
	cy 18	9.26	102.09	7.00	0.072	118.42	63.67	182.09	0.7298
	cy 19	7.41	101.01	7.32	0.072	115.81	67.97	183.78	0.7663
	cy 20	8.29	101.53	7.23	0.069	117.12	67.76	184.88	0.6739
	cy 41	8.63	103.88	7.66	0.097	120.27	74.17	194.44	0.6851
	cy 42	13.19	100.26	6.99	0.099	120.54	81.25	201.79	-
	cy 43	8.53	105.94	6.82	0.110	121.40	70.65	192.05	0.6837
	cy 44	15.28	104.90	8.34	0.092	128.61	66.67	195.28	0.6560
	cy 45	19.30	101.27	7.83	0.093	128.49	77.88	206.37	-
	cy 54	12.21	101.03	7.35	0.103	120.69	82.38	203.07	-
	cy 55	14.20	101.57	8.20	0.114	124.08	73.54	197.62	-
	cy 56	17.75	98.83	7.60	0.109	124.29	-	-	-
	cy 57	15.21	100.48	7.51	0.102	123.30	71.89	195.19	0.6744
	cy 58	13.01	102.65	6.15	0.101	121.91	73.03	194.94	0.6894
	cy 69	17.13	98.24	7.57	0.115	123.06	74.44	197.50	-
	cy 70	22.38	97.18	7.74	0.139	127.44	76.61	204.05	-
	cy 71	13.84	97.69	7.48	0.119	119.13	66.57	185.70	-
15:00 h	cy 1	9.01	95.44	5.45	0.047	109.95	67.36	177.31	0.7154
	cy 2	13.22	105.70	7.63	0.055	126.61	69.51	196.12	0.6991
	cy 3	10.14	103.56	6.96	0.041	120.70	71.75	192.45	0.7277
	cy 4	11.92	95.35	5.90	0.505	113.68	72.09	185.77	0.7347
	cy 5	15.89	100.28	2.67	0.066	118.91	66.98	185.89	0.7210
	cy 21	11.00	96.21	6.65	0.068	113.93	72.82	186.75	-
	cy 22	15.09	96.74	7.75	0.070	119.65	68.10	187.75	0.7398
	cy 23	11.51	96.19	6.13	0.085	113.92	79.90	193.82	0.7417
	cy 24	8.30	103.69	7.32	0.062	119.37	76.07	195.44	0.7010
	cy 25	16.83	98.87	6.68	0.092	122.47	77.78	200.25	0.7107
	cy 26	14.25	101.53	8.23	0.056	124.07	80.54	204.61	0.7229
	cy 27	19.82	103.65	8.14	0.065	131.68	84.71	216.39	0.7157
	cy 28	14.49	102.59	6.78	0.079	123.94	78.31	202.25	-
	cy 29	18.94	104.15	6.95	0.121	130.16	79.75	209.91	0.7009
	cy 30	13.94	105.17	8.31	0.052	127.47	75.92	203.39	0.7279
	cy 46	14.40	97.17	7.50	0.076	119.15	73.70	192.85	0.6835
	cy 47	17.99	100.26	7.58	0.091	125.92	73.90	199.82	-
	cy 48	15.26	100.76	8.17	0.094	124.28	74.96	199.24	0.6806
21:00 h	cy 6	10.38	95.98	2.66	0.143	109.16	67.60	176.76	-
	cy 7	6.05	110.54	7.63	0.061	124.28	73.14	197.42	0.7512
	cy 8	5.87	100.31	7.50	0.400	114.08	67.77	181.85	0.7326
	cy 9	5.88	104.09	8.38	0.066	118.42	69.05	187.47	0.7037
	cy 10	3.71	107.85	7.80	0.060	119.42	71.17	190.59	0.6938
	cy 31	9.30	107.23	8.91	0.050	125.49	74.58	200.07	0.6845
	cy 32	9.10	105.69	8.23	0.077	123.10	83.14	206.24	0.6520
	cy 33	11.23	102.11	7.63	0.067	121.04	71.08	192.12	0.6773
	cy 34	13.40	102.62	7.37	0.085	123.48	74.85	198.33	-
	cy 35	11.18	102.62	7.29	0.082	121.17	73.30	194.47	0.6686
	cy 49	14.59	97.78	7.51	0.090	119.97	69.65	189.62	0.6776
	cy 50	18.80	92.85	7.42	0.103	119.17	71.88	191.05	-
	cy 51	20.25	95.57	7.93	0.119	123.87	75.79	199.66	0.6127
	cy 52	16.67	95.58	7.52	0.099	119.87	73.91	193.78	-
	cy 53	12.58	97.20	6.58	0.299	116.66	84.71	201.37	0.6619
	cy 64	11.66	105.61	7.74	0.176	125.19	62.10	187.29	-
	cy 65	16.44	101.40	8.51	0.118	126.47	65.31	191.78	-
	cy 66	12.20	97.18	6.53	0.153	116.06	68.37	184.43	0.6376

APPENDIX

TABLE 22 : Erythrocytic electrolyte, total cation and total cation + Cl^- raw data for cell H_2O in goldfish acclimated to 20°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-
		(mmol/l cell H_2O)						
03:00 h	203	24.18	154.08	13.38	0.379	192.02	93.08	285.10
	204	17.56	125.10	10.11	1.124	153.89	79.97	233.86
	2015	-	-	-	-	-	-	-
	2016	19.37	129.52	12.77	0.643	162.30	105.66	267.96
	2021	-	-	-	-	-	80.89	-
	2022	16.08	140.21	9.54	0.367	166.20	98.42	264.62
	2023	16.12	146.97	11.52	0.351	174.96	93.83	268.79
	2024	10.54	151.35	10.91	0.419	173.22	93.14	266.36
	2025	25.76	159.79	11.90	0.422	197.87	104.60	302.47
	2026	8.82	161.05	12.25	0.455	182.58	98.93	281.51
	2027	18.49	170.03	12.45	0.402	201.37	114.47	315.84
	2028	8.27	146.01	10.26	0.959	165.50	137.73	303.23
	2029	12.92	141.10	10.53	0.948	165.50	112.14	277.64
	2030	12.03	159.80	11.58	0.476	183.89	107.39	291.28
	2031	20.93	141.41	11.31	0.456	174.11	99.96	274.07
	2032	23.80	147.24	11.71	0.478	183.23	-	-
	2033	24.52	137.07	11.47	0.734	173.79	103.71	277.50
	2034	35.69	98.97	10.96	0.866	146.49	-	-
	2035	21.65	148.04	12.35	0.275	182.32	82.71	265.03
09:00 h	205	24.64	137.59	11.12	0.641	173.99	-	-
	206	-	-	-	-	-	-	-
	2017	-	-	-	-	-	79.44	-
	2018	16.28	138.19	11 50	0.783	166.75	105.65	272.40
	2036	21.31	194.34	13.57	-	-	138.72	-
	2037	10.39	151.28	10.52	0.432	172.62	96.36	268.98
	2038	24.87	166.81	9.59	0.114	201.38	108.91	310.29
	2039	22.38	157.14	9.69	0.472	189.68	-	-
	2040	21.90	163.70	11.14	0.424	197.16	111.23	308.39
	2041	19.68	165.63	12.01	0.646	197.97	117.84	315.81
	2047	20.12	140.03	12.52	0.412	173.08	104.29	277.37
	2048	16.45	150.72	11.03	0.458	178.66	-	-
	2049	9.45	141.33	9.77	0.376	160.93	106.28	267.21
	2050	16.98	143.59	11.13	0.391	172.09	100.69	272.78
	2051	17.75	188.41	14.41	0.651	221.22	142.85	364.07
	2052	11.66	149.92	11.76	0.446	173.79	84.81	258.60
	2070	16.22	141.68	12.54	0.412	170.85	104.48	275.33
	2071	16.57	157.12	14.08	0.414	188.18	107.56	295.74
15:00 h	207	16.04	142.02	14.09	0.489	172.64	97.78	270.42
	208	26.31	170.84	14.00	2.297	213.45	119.54	332.99
	2011	18.05	136.88	9.11	1.077	165.12	99.55	264.67
	2012	-	-	-	-	-	-	-
	2019	-	-	-	-	-	-	-
	2020	-	-	11.37	0.558	-	-	-
	2053	10.47	129.30	8.02	0.441	148.23	107.59	255.82
	2054	15.10	134.57	11.75	0.304	161.72	100.65	262.37
	2055	13.45	145.93	9.23	0.379	168.99	116.23	285.22
	2056	9.22	147.60	10.17	0.385	167.38	106.67	274.05
	2057	11.61	147.26	10.35	0.387	169.61	110.15	279.76
	2063	19.14	181.80	14.66	0.544	216.14	151.32	367.46
	2064	12.21	152.85	11.95	0.484	177.49	-	-
	2065	19.62	145.82	11.45	0.384	177.27	121.18	298.45
	2066	14.22	146.73	11.76	0.355	173.07	113.57	286.64
	2067	20.60	179.05	15.06	0.485	215.20	148.50	363.70
	2072	17.90	146.16	13.62	0.379	177.68	96.97	274.65
	2073	21.03	157.66	13.94	0.405	193.04	-	-
21:00 h	201	21.05	142.07	10.18	0.661	173.96	-	-
	202	-	-	-	-	-	92.54	-
	209	7.95	134.39	9.94	0.504	152.78	-	-
	2010	9.81	118.90	9.34	0.359	138.41	92.92	231.33
	2013	12.34	125.32	10.53	0.482	148.67	-	-
	2014	11.33	130.28	10.57	0.487	152.67	107.24	259.91
	2042	21.70	149.71	12.89	0.407	184.71	121.89	306.60
	2043	9.08	141.53	10.97	0.470	162.05	96.34	258.39
	2044	16.63	157.51	10.24	0.489	184.87	113.69	298.56
	2045	18.77	140.89	11.07	0.402	171.13	105.49	276.62
	2046	18.49	165.02	12.33	0.456	196.30	119.37	315.67
	2058	19.24	140.35	12.27	0.399	172.26	118.71	290.97
	2059	12.64	117.94	9.11	0.360	140.05	96.38	236.43
	2060	15.78	145.69	10.28	0.514	172.26	115.81	288.07
	2061	17.42	137.30	11.08	0.446	166.25	103.20	269.45
	2062	21.21	135.66	10.89	0.480	168.24	121.80	290.04
	2068	18.24	140.94	13.90	0.399	173.48	91.66	265.14
	2069	16.96	149.39	13.74	0.441	180.53	96.61	277.14

APPENDIX

TABLE 23 : Erythrocytic electrolyte, total cation and total cation + Cl^- raw data for cell H_2O in goldfish acclimated to 25°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-
		(mmol/l cell H2O)						
03:00 h	254	25.21	137.53	12.49	0.379	175.61	118.48	294.09
	255	43.44	112.06	7.15	0.618	163.27	122.22	285.49
	256	16.41	138.81	9.13	0.364	164.71	109.63	274.34
	2522	9.25	147.17	11.87	0.519	168.81	121.79	290.60
	2523	16.01	142.57	11.60	0.395	170.58	107.07	277.65
	2524	-	-	-	-	-	-	-
	2535	31.32	132.29	8.98	-	-	107.29	-
	2536	37.74	140.00	-	-	-	96.46	-
	2537	21.64	133.52	13.30	-	-	89.77	-
	2564	14.62	149.01	10.06	0.133	173.82	107.68	281.50
	2565	14.27	14[illegible].76	11.48	0.324	169.83	71.20	241.03
	2566	29.22	152.18	13.42	0.443	195.26	122.24	317.50
	2567	-	- -	-	-	-	111.45	-
	2568	21.72	141.38	10.52	0.278	173.90	101.62	275.52
	2569	25.00	134.03	11.55	0.267	170.85	78.71	249.56
	2570	18.01	133.77	9.38	0.224	161.38	71.37	232.75
	2571	20.82	139.89	10.71	0.307	171.73	65.10	236.83
09:00 h	257	21.20	134.90	9.28	0.469	165.85	107.70	273.55
	258	-	-	-	-	-	-	-
	259	-	-	-	-	-	-	-
	2525	27.39	124.05	9.30	0.479	161.22	113.97	275.19
	2526	-	-	-	-	-	116.42	-
	2527	12.42	132.34	3.97	0.360	149.09	109.84	258.93
	2538	31.23	140.69	10.15	0.200	182.27	95.83	278.10
	2539	29.80	135.00	9.39	-	-	104.60	-
	2540	-	-	-	0.273	-	92.64	-
	2544	16.91	135.41	7.79	0.205	160.32	102.87	263.19
	2545	23.56	129.26	11.07	0.157	164.05	118.85	282.90
	2546	21.37	136.16	10.12	0.169	167.82	109.97	277.79
	2547	18.93	128.20	10.25	0.046	157.43	105.35	262.78
	2548	23.08	121.21	9.59	0.169	154.05	97.99	252.04
	2549	24.30	115.43	10.10	0.195	150.03	119.63	269.66
	2550	27.20	130.33	12.00	0.350	169.88	118.37	288.25
	2551	32.67	130.54	14.44	0.118	177.77	118.88	296.65
	2552	27.15	142.85	9.57	0.156	179.73	120.49	300.22
	2553	28.84	139.65	10.85	0.157	179.50	125.28	304.78
15:00 h	2510	13.13	128.12	7.85	0.216	149.32	118.58	267.90
	2511	24.53	141.18	11.18	0.479	177.37	-	-
	2512	13.39	127.42	10.83	-	-	108,91	-
	2516	18.34	137.65	9.97	-	-	-	-
	2517	19.14	138.85	11.27	0.609	169.87	120.86	290.73
	2518	-	-	-	-	-	-	-
	2528	18.61	134.14	11.22	0.240	164.21	111.97	276.18
	2529	21.68	135.55	11.64	0.468	169.34	116.48	285.82
	2530	11.73	45.55	3.62	-	-	88.53	-
	2531	10.96	143.02	9.55	-	-	97.43	--
	2541	25.02	142.48	9.44	-	-	98.09	-
	2542	28.63	132.08	9.85	-	-	107.38	-
	2543	23.73	134.97	10.02	-	-	105.75	-
	2554	11.89	133.27	8.22	0.101	153.48	118.15	271.63
	2555	19.81	137.43	9.30	0.207	166.75	126.53	293.28
	2556	18.09	122.33	9.13	0.136	149.69	103.02	252.71
	2557	19.27	125.40	9.92	0.029	154.62	109.76	264.38
	2558	8.55	150.83	8.67	0.109	168.16	114.13	282.29
21:00 h	251	12.76	119.48	9.39	0.565	142.20	112.40	254.60
	252	-	-	-	-	-	-	-
	253	30.74	129.55	9.90	0.374	170.56	117.70	288.26
	2513	14.30	140.01	11.22	0.521	166.05	107.51	273.56
	2514	22.93	140.76	10.99	0.395	175.08	114.19	289.27
	2515	21.26	139.24	9.55	0.261	170.31	122.33	292.64
	2519	19.25	134.45	10.20	0.511	164.41	128.07	292.48
	2520	20.87	122.92	10.97	0.346	155.11	116.72	271.83
	2521	12.64	128.70	10.87	0.395	152.61	-	-
	2532	34.33	141.27	8.97	-	-	117.75	-
	2533	52.30	208.05	15.05	0.512	275.91	134.01	409.92
	2534	27.32	138.34	9.25	-	-	102.11	-
	2559	-	-	-	-	-	-	-
	2560	11.27	165.16	10.61	0.134	187.17	120.16	307.33
	2561	16.36	170.05	10.74	0.154	197.30	111.64	308.94
	2562	11.95	153.11	11.05	0.166	176.28	111.61	287.89
	2563	14.56	151.85	10.24	0.103	176.75	118.00	294.75

APPENDIX

TABLE 24 : Erythrocytic electrolyte, total cation and total cation + Cl^- raw data for cell H_2O in goldfish acclimated to 30^oC.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-
		(mmol/l cell H_2O)						
03:00 h	3011	27.08	139.14	9.03	0.185	175.44	114.59	290.03
	3012	13.26	134.88	11.11	0.095	159.35	103.17	262.52
	3013	22.19	135.11	9.50	0.202	167.00	109.76	276.76
	3014	22.24	141.29	8.42	0.144	172.09	104.66	276.75
	3015	28.10	152.16	9.36	-	-	123.04	-
	3032	25.63	129.28	8.70	-	-	102.30	-
	3033	37.23	141.25	10.23	0.035	188.75	113.88	302.63
	3034	25.65	131.23	7.77	-	-	104.11	-
	3035	29.97	141.27	9.12	0.120	180.48	103.91	284.39
	3059	26.26	155.66	9.60	0.101	191.62	103.29	294.91
	3060	20.22	152.44	10.31	0.084	183.05	98.82	281.87
	3061	21.02	157.68	9.59	0.099	188.39	108.08	296.47
	3062	19.73	160.83	11.23	0.173	191.96	108.74	300.70
	3063	21.68	150.60	7.77	0.140	180.19	111.60	291.79
	3064	14.00	156.01	8.87	0.147	179.03	118.39	297.42
09:00 h	3016	-	-	-	-	-	112.89	-
	3017	26.95	132.34	8.80	0.013	168.10	110.80	278.90
	3018	23.49	140.55	9.79	0.175	174.01	118.13	292.14
	3019	21.18	145.09	8.35	0.191	174.81	110.97	285.78
	3036	32.54	114.56	8.76	0.301	156.16	112.13	268.29
	3037	31.08	149.73	11.32	0.096	192.23	105.20	297.43
	3038	33.23	145.95	9.10	-	-	116.68	-
	3039	30.80	136.47	8.95	0.119	176.34	114.01	290.35
	3040	34.46	138.48	10.68	0.036	183.66	116.33	299.99
	3065	20.48	164.52	10.41	0.143	195.55	121.31	316.86
	3066	17.84	151.35	9.04	0.135	178.37	115.42	293.79
	3067	18.09	159.29	8.44	0.136	185.96	121.01	306.97
	3068	11.01	169.39	9.05	0.162	189.61	127.57	317.18
	3069	16.32	153.99	10.25	0.139	180.70	119.40	300.10
	3070	17.89	159.05	11.37	0.153	188.46	114.33	302.79
15:00 h	301	16.97	142.68	8.50	0.088	168.24	122.28	290.52
	302	42.38	197.30	10.37	1.101	251.15	123.54	374.69
	303	30.30	143.80	9.16	0.193	183.45	106.71	290.16
	304	24.41	145.24	9.97	-	-	101.32	-
	305	20.63	143.94	9.25	-	-	108.70	-
	3020	17.38	127.65	10.87	0.205	156.11	113.22	269.33
	3021	29.73	136.09	8.27	0.102	174.19	114.64	288.83
	3022	35.28	151.89	11.60	0.072	198.84	118.93	317.77
	3023	19.96	122.51	9.71	0.092	152.27	107.94	260.21
	3024	19.19	134.32	9.03	0.090	162.63	132.10	294.73
	3025	21.17	116.24	12.65	0.450	150.51	102.26	252.77
	3026	26.79	137.10	10.85	0.561	175.30	103.92	279.22
	3027	12.39	142.53	9.75	0.068	164.74	102.14	266.88
	3041	35.04	124.46	7.78	0.429	167.71	114.03	281.74
	3042	26.77	136.69	10.20	0.047	173.71	105.08	278.79
	3043	29.47	132.46	10.89	0.447	173.27	112.96	286.23
	3044	30.19	131.25	9.97	0.746	172.16	112.74	284.90
	3045	35.84	137.25	10.43	0.075	183.60	112.97	296.57
	3046	26.63	141.33	8.58	0.097	176.64	105.32	281.96
	3047	17.72	144.18	8.06	0.042	170.00	112.45	282.45
	3048	23.29	145.00	7.72	0.017	176.03	144.42	290.45
	3049	18.00	150.57	8.50	0.046	177.12	125.68	302.80
	3050	29.23	144.68	9.65	0.059	183.62	118.43	302.05
	3051	40.85	135.79	8.53	0.091	185.26	120.21	305.47
	3052	18.59	152.76	9.92	0.119	181.39	102.42	283.81
21:00 h	306	22.92	133.89	8.96	0.090	165.86	115.74	281.60
	307	14.81	140.62	8.28	0.080	163.79	113.26	277.05
	308	30.09	141.88	10.22	0.026	182.22	115.51	297.73
	309	33.58	157.51	10.53	0.387	202.01	108.84	310.85
	3010	22.49	137.56	9.30	0.094	169.44	111.19	280.63
	3028	31.14	114.25	10.16	0.380	155.93	92.12	248.05
	3029	32.09	141.81	11.78	0.226	185.91	-	-
	3030	24.91	131.10	8.79	0.135	164.94	111.28	276.22
	3031	33.13	139.95	10.96	0.338	184.38	-	-
	3053	18.65	148.28	9.50	0.086	176.52	134.86	311.38
	3054	22.17	134.49	9.22	0.097	165.98	98.86	264.84
	3055	17.54	160.51	11.52	0.124	189.69	112.66	302.35
	3056	15.93	153.24	10.00	0.095	179.27	108.73	288.00
	3057	18.85	151.93	9.87	0.107	180.76	132.02	312.78
	3058	19.26	146.53	9.72	0.091	175.60	94.31	269.91

APPENDIX

TABLE 25 : Erythrocytic electrolyte, total cation and total cation + Cl^- raw data for cell H_2O in goldfish acclimated to a cycling temperature of 25°± 5°C.

SAMPLING TIME	CODE NO	Na^+	K^+	$Mg2^+$	$Ca2^+$	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-
		(mmol/l cell H_2O)						
03:00 h	cy 11	12.41	134.77	9.27	0.081	156.53	93.27	249.80
	cy 12	-	143.27	8.11	0.084	-	100.89	-
	cy 13	14.40	135.96	9.69	0.200	160.25	94.24	254.49
	cy 14	7.48	139.03	9.49	0.155	156.16	89.04	245.20
	cy 15	11.37	146.30	12.11	0.110	169.89	87.01	256.90
	cy 36	14.80	146.78	11.74	0.104	173.42	-	-
	cy 37	10.18	162.20	11.48	0.161	184.02	117.55	301.57
	cy 38	12.31	162.32	11.15	0.156	185.94	112.46	298.40
	cy 39	13.00	156.28	12.43	0.137	181.85	101.11	282.96
	cy 40	12.49	156.26	12.26	0.114	181.12	115.47	286.59
	cy 59	7.95	152.29	8.60	0.150	168.99	112.97	281.96
	cy 60	8.74	158.58	8.89	0.164	176.37	111.32	287.69
	cy 61	-	-	-	-	-	107.55	-
	cy 62	11.55	156.42	11.51	0.176	179.66	102.50	282.16
	cy 63	11.82	155.47	11.42	0.172	178.88	100.58	279.46
	cy 67	20.58	153.73	11.94	0.218	186.47	99.20	285.67
	cy 68	14.08	149.89	11.21	0.259	175.44	108.34	283.78
09:00 h	cy 16	9.25	137.05	7.72	0.100	154.12	100.11	254.23
	cy 17	15.91	148.83	11.14	0.111	175.99	98.21	274.20
	cy 18	12.69	139.89	9.59	0.099	162.27	87.24	249.51
	cy 19	9.67	131.82	9.55	0.094	151.13	88.70	239.83
	cy 20	12.30	150.66	10.73	0.102	173.79	100.55	274.34
	cy 41	12.60	151.63	11.18	0.142	175.55	108.26	283.81
	cy 42	18.81	143.00	9.97	0.141	171.92	115.89	287.81
	cy 43	12.48	154.95	9.98	0.161	177.57	103.33	280.90
	cy 44	23.29	159.91	12.71	0.140	196.05	101.63	297.68
	cy 45	27.53	144.44	11.17	0.133	183.27	111.08	294.35
	cy 54	17.42	144.10	10.48	0.147	172.15	117.50	289.65
	cy 55	20.25	144.87	11.70	0.163	176.98	104.89	281.87
	cy 56	25.32	140.96	10.84	0.155	177.28	-	-
	cy 57	22.55	148.99	11.14	0.151	182.83	106.60	289.43
	cy 58	18.87	148.90	8.92	0.147	176.84	105.93	282.77
	cy 69	24.43	140.12	10.80	0.164	175.51	106.18	281.69
	cy 70	31.92	138.61	11.04	0.198	181.77	109.27	291.04
	cy 71	19.74	139.34	10.67	0.170	169.92	94.95	264.87
15:00 h	cy 1	12.59	133.41	7.62	0.066	153.69	94.16	247.85
	cy 2	18.91	151.19	10.91	0.079	181.09	99.43	280.52
	cy 3	13.93	142.31	9.56	0.056	165.86	98.60	264.46
	cy 4	16.22	129.78	8.03	0.687	154.72	98.12	252.84
	cy 5	22.04	139.08	3.70	0.092	164.91	93.90	257.81
	cy 21	15.39	134.60	9.30	0.095	159.39	101.87	261.26
	cy 22	20.40	130.77	10.48	0.095	161.75	92.05	253.80
	cy 23	15.52	129.69	8.26	0.115	153.59	107.73	261.32
	cy 24	11.84	147.92	10.44	0.088	170.29	108.52	278.81
	cy 25	23.68	139.12	9.40	0.129	172.33	109.44	281.77
	cy 26	19.71	140.45	11.38	0.077	171.62	111.41	283.03
	cy 27	27.69	144.82	11.37	0.091	183.97	118.36	302.33
	cy 28	20.27	143.52	9.49	0.111	173.39	109.56	282.95
	cy 29	27.02	148.59	9.92	0.173	185.70	113.78	299.48
	cy 30	19.15	144.48	11.42	0.071	175.12	104.30	279.42
	cy 46	21.07	142.17	10.97	0.111	174.32	107.83	282.15
	cy 47	25.17	140.26	10.60	0.127	176.16	103.39	279.55
	cy 48	22.42	148.05	12.00	0.138	182.61	110.14	292.75
21:00 h	cy 6	15.28	141.25	3.91	0.210	160.65	99.48	260.13
	cy 7	8.05	147.15	10.16	0.081	165.44	97.36	262.80
	cy 8	8.01	136.92	10.24	0.546	155.72	92.51	248.23
	cy 9	8.36	147.92	11.91	0.094	168.28	98.12	266.40
	cy 10	5.35	155.45	11.24	0.086	172.13	102.58	274.71
	cy 31	13.59	156.65	13.02	0.073	183.33	108.96	292.29
	cy 32	13.96	162.10	12.62	0.118	188.80	127.52	316.32
	cy 33	16.58	150.76	11.27	0.099	178.71	104.95	283.66
	cy 34	19.72	151.02	10.85	0.125	181.72	110.15	291.87
	cy 35	16.72	153.48	10.90	0.123	181.22	109.63	290.85
	cy 49	21.53	144.30	11.08	0.133	177.04	102.79	279.83
	cy 50	27.67	136.64	10.92	0.152	173.38	105.78	281.16
	cy 51	33.05	155.98	12.94	0.194	202.16	123.70	325.86
	cy 52	24.53	140.66	11.07	0.146	176.41	108.77	285.18
	cy 53	19.01	146.85	9.94	0.452	176.25	127.98	304.23
	cy 64	17.16	155.42	11.39	0.259	184.23	91.39	275.62
	cy 65	24.19	149.23	12.52	0.174	186.11	96.11	282.22
	cy 66	19.13	152.42	10.24	0.240	182.03	107.23	289.26

APPENDIX

TABLE 26 : Muscle water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 20°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (kg/kg)
		(mmol/kg)							
03:00 h	203	26.46	110.69	13.66	10.06	160.87	14.7	175.58	0.7995
	204	20.60	120.03	13.64	7.24	161.51	10.42	171.93	0.7857
	2015	18.39	114.75	15.23	6.29	154.66	8.29	162.95	0.7859
	2016	25.69	123.00	13.11	5.58	167.38	11.88	179.26	0.7985
	2021	30.61	97.00	12.24	13.70	153.55	13.56	167.11	0.7986
	2022	23.76	117.22	12.71	8.80	162.49	11.92	174.41	0.7886
	2023	21.29	110.17	13.22	8.10	152.78	12.00	164.78	0.7950
	2024	24.13	106.52	11.96	8.10	150.71	14.17	164.88	0.8000
	2025	18.59	108.71	-	7.13	-	9.60	-	0.7937
	2026	25.29	105.56	12.20	2.96	146.01	11.94	157.95	0.7919
	2027	22.98	109.92	13.54	9.22	155.66	12.35	168.01	0.7874
	2028	95.74	-	4.17	7.36	-	-	-	0.8800
	2029	30.97	38.85	10.18	7.22	87.22	15.96	103.18	0.8014
	2030	30.10	100.76	12.59	2.88	146.33	15.45	161.78	0.7975
	2031	35.29	49.28	8.69	6.10	99.36	17.76	117.12	0.8151
	2032	34.46	48.26	9.05	10.93	102.70	15.87	118.57	0.8152
	2033	67.56	11.54	5.71	15.37	100.18	-	-	0.8490
	2034	141.49	-	-	3.71	-	-	-	0.8823
	2035	30.04	29.22	9.42	3.77	72.45	11.20	83.65	0.8135
09:00 h	205	23.34	123.66	14.24	5.81	167.05	13.14	180.19	0.7877
	206	24.30	122.52	14.10	10.74	171.66	12.01	183.67	0.7890
	2017	17.51	128.51	16.27	5.01	167.30	9.39	176.69	0.7685
	2018	19.39	125.60	15.58	5.95	166.52	10.56	177.08	0.7764
	2036	21.46	105.35	13.29	3.28	143.38	11.95	155.33	0.7909
	2037	28.57	95.34	12.90	6.35	143.16	17.85	161.01	0.7915
	2038	24.16	90.16	12.35	3.15	129.82	15.11	144.93	0.7879
	2039	32.34	61.12	11.21	4.43	109.10	18.10	127.20	0.7969
	2040	70.10	-	6.81	8.20	-	-	-	0.8372
	2041	29.08	94.66	13.64	4.67	142.05	17.27	159.32	0.7842
	2047	18.36	108.11	12.34	2.73	141.63	12.58	154.21	0.7810
	2048	19.89	77.75	12.60	3.45	113.69	15.89	128.58	0.7911
	2049	17.99	90.30	13.80	2.81	124.90	13.52	138.42	0.7702
	2050	18.05	104.59	13.46	6.52	142.62	12.25	154.87	0.7929
	2051	22.13	94.46	13.62	3.65	133.86	14.89	148.75	0.7792
	2052	21.38	106.71	12.75	3.01	143.85	13.16	157.01	0.7946
	2070	21.87	95.07	11.74	3.46	132.14	13.25	145.39	0.7898
	2071	20.94	105.09	12.12	2.67	140.82	14.57	155.39	0.7869
15:00 h	207	17.57	121.80	16.26	5.94	161.57	8.94	170.51	0.7716
	208	22.08	120.38	15.35	6.26	164.07	10.96	175.03	0.7790
	2011	18.94	114.78	15.15	8.50	157.37	9.83	167.20	0.7815
	2012	29.36	100.81	12.96	5.57	148.70	14.89	163.59	0.7960
	2019	22.90	102.70	13.64	12.32	151.56	12.30	163.86	0.7910
	2020	16.80	114.75	15.55	6.10	153.20	8.35	161.55	0.7672
	2053	19.30	88.28	13.79	3.48	124.85	13.05	137.90	0.7779
	2054	16.24	108.87	12.96	2.82	140.89	12.03	152.92	0.7923
	2055	22.50	96.51	13.77	4.91	137.59	13.75	151.34	0.7868
	2056	19.33	114.97	14.01	3.51	151.82	12.24	164.06	0.7882
	2057	24.74	107.53	13.15	5.01	150.43	15.82	166.25	0.7970
	2063	19.23	100.85	11.77	2.96	134.81	13.84	148.65	0.7984
	2064	15.81	98.18	12.23	2.91	129.13	12.26	141.39	0.7860
	2065	16.53	103.17	13.08	2.53	135.31	11.58	146.89	0.7874
	2066	19.68	81.08	10.91	2.74	114.41	13.46	127.87	0.7777
	2067	17.67	97.01	11.81	3.28	129.77	13.18	142.95	0.7853
	2072	19.00	100.54	12.55	2.66	134.75	14.17	148.92	0.7809
	2073	23.99	68.45	10.86	4.15	107.45	17.76	125.21	0.7927
21:00 h	201	23.01	123.23	13.23	11.96	171.49	12.56	184.05	0.7979
	202	18.24	116.97	15.15	9.43	159.79	9.13	168.92	0.7835
	209	17.78	117.27	16.40	5.63	157.08	8.69	165.77	0.7679
	2010	18.10	123.95	17.41	5.16	164.62	9.19	173.81	0.7553
	2013	20.45	120.79	14.62	7.38	163.24	11.96	175.20	0.7799
	2014	19.70	121.99	16.12	7.68	165.49	9.73	175.22	0.7663
	2042	19.96	109.71	13.31	4.21	147.19	11.88	159.07	0.7898
	2043	24.13	87.95	13.41	7.65	133.14	14.65	147.79	0.7913
	2044	21.08	106.02	13.56	4.38	145.04	13.17	158.21	0.7848
	2045	23.98	95.55	12.72	3.42	135.67	15.31	150.98	0.7927
	2046	16.77	90.03	12.55	2.24	121.59	15.00	136.59	0.7708
	2058	21.10	104.72	13.00	3.12	141.94	12.21	154.15	0.7815
	2059	15.17	95.38	12.88	2.80	126.23	11.06	137.29	0.7800
	2060	18.87	92.32	12.80	2.95	126.94	13.34	140.28	0.7873
	2061	18.93	103.37	12.75	2.83	137.88	12.27	150.15	0.7783
	2062	18.71	102.53	12.66	2.80	136.70	12.46	149.16	0.7855
	2068	22.07	99.76	12.30	2.93	137.06	14.44	151.50	0.7847
	2069	21.92	100.19	11.77	3.27	137.15	14.61	151.76	0.7924

APPENDIX

TABLE 27 : Muscle water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 25°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS	H_2O CONTENT
		(mmol/kg)							(kg/kg)
03:00 h	254	18.76	119.10	15.09	10.84	163.79	8.83	172.62	0.7902
	255	45.03	54.01	13.83	20.28	133.15	18.72	151.87	0.8038
	256	23.16	109.67	16.77	12.76	162.36	11.92	174.28	0.7773
	2522	24.20	122.99	15.59	12.03	174.81	10.10	184.91	0.7802
	2523	26.25	124.79	16.00	12.46	179.50	12.33	191.83	0.7865
	2524	37.52	111.79	14.28	22.24	185.83	16.03	201.86	0.7992
	2535	9.73	116.59	11.87	10.65	148.84	8.19	157.03	0.7849
	2536	11.31	114.39	14.02	9.47	149.19	8.10	157.29	0.7829
	2537	17.70	95.10	11.21	10.25	134.26	13.19	147.45	0.8085
	2564	25.59	108.80	14.83	4.34	153.56	12.31	165.87	0.7715
	2565	28.05	109.25	13.29	3.86	154.45	12.90	167.35	0.7556
	2566	25.29	108.45	14.32	3.99	152.05	11.98	164.03	0.7790
	2567	26.92	100.90	14.80	4.56	147.18	12.35	159.53	0.7769
	2568	32.82	95.75	18.38	7.60	154.55	14.36	168.91	0.7567
	2569	32.48	80.63	14.02	3.56	130.69	12.00	142.69	0.7737
	2570	29.04	100.59	13.29	3.21	146.13	13.05	159.18	0.7628
	2571	31.32	98.51	14.06	3.44	147.33	14.58	161.91	0.7746
09:00 h	257	27.42	117.78	15.17	15.27	175.64	12.11	187.75	0.7910
	258	31.27	99.70	16.76	10.46	158.19	13.96	172.15	0.7548
	259	39.66	72.23	14.11	20.14	146.14	17.51	163.65	0.8086
	2525	33.33	123.13	15.54	24.35	196.35	13.45	209.80	0.7845
	2526	28.83	123.15	16.17	11.89	180.04	11.14	191.18	0.7875
	2527	24.79	120.30	15.90	7.92	168.91	10.91	179.82	0.7870
	2538	14.16	112.14	13.38	11.90	151.58	9.95	161.53	0.7914
	2539	22.58	106.62	11.70	7.55	148.45	14.09	162.54	0.8109
	2540	14.70	115.03	11.86	16.24	157.83	10.29	168.12	0.7925
	2544	27.48	117.20	15.21	12.24	172.13	11.65	183.78	0.7951
	2545	23.75	120.70	15.05	6.20	165.70	10.76	176.46	0.7846
	2546	25.35	123.81	13.18	9.93	172.27	11.58	183.85	0.7970
	2547	32.53	115.19	13.67	10.82	172.21	11.75	183.96	0.7873
	2548	38.99	109.93	11.80	4.70	165.42	20.39	185.81	0.8133
	2549	29.52	114.70	14.95	9.79	168.96	11.78	180.74	0.7873
	2550	35.13	98.46	13.14	17.74	164.47	13.19	177.66	0.7983
	2551	28.87	107.44	13.00	8.96	158.27	10.60	168.87	0.7920
	2552	27.01	107.31	13.55	12.83	160.70	12.96	173.66	0.8040
	2553	26.91	104.88	12.87	10.12	154.78	12.90	167.68	0.8040
15:00 h	2510	34.20	105.85	18.14	12.84	171.03	15.09	186.12	0.7610
	2511	40.33	119.98	14.82	14.58	189.71	13.14	202.85	0.7787
	2512	26.66	120.30	15.23	15.01	177.20	10.00	187.20	0.7819
	2516	40.87	84.84	15.37	16.46	157.54	14.55	172.09	0.7701
	2517	31.92	116.84	15.94	12.76	177.46	11.33	188.79	0.7805
	2518	36.75	104.40	14.00	16.05	171.20	12.81	184.01	0.8033
	2528	28.58	125.75	16.88	9.23	180.44	9.43	189.87	0.7758
	2529	14.24	114.14	16.62	9.98	154.98	8.52	163.50	0.7741
	2530	14.41	109.39	13.12	9.07	145.99	9.87	155.86	0.7852
	2531	16.04	105.22	13.41	11.71	146.38	9.55	155.93	0.7877
	2541	12.17	119.92	14.65	14.53	161.27	8.55	169.82	0.7835
	2542	23.88	115.30	13.09	12.47	164.74	11.37	176.11	0.8070
	2543	20.94	117.41	13.09	10.18	161.62	12.16	173.78	0.8082
	2554	16.38	111.40	15.10	4.33	147.21	8.97	156.18	0.7815
	2555	24.06	118.30	14.04	4.82	161.22	9.97	171.19	0.7918
	2556	15.72	107.82	13.82	6.08	143.44	10.86	154.30	0.7849
	2557	21.18	108.17	14.62	5.23	149.20	9.82	159.02	0.7805
	2558	17.13	113.30	14.20	4.26	148.89	10.51	159.40	0.7908
21:00 h	251	25.78	122.08	16.92	16.08	180.86	10.26	191.12	0.7699
	252	39.29	113.80	15.28	12.89	181.26	18.35	199.61	0.7936
	253	29.31	113.97	14.71	13.01	171.00	14.46	185.46	0.7912
	2513	24.71	124.70	14.33	10.51	174.25	10.03	184.28	0.7889
	2514	27.71	118.04	14.87	11.99	172.61	10.30	182.91	0.7911
	2515	27.20	119.97	15.27	19.85	182.29	10.38	192.67	0.7791
	2519	37.44	97.09	15.25	17.26	167.04	13.57	180.61	0.7797
	2520	34.48	121.98	17.07	12.87	186.40	11.69	198.09	0.7479
	2521	31.73	118.73	14.64	16.76	181.86	11.51	193.37	0.8015
	2532	20.87	167.47	19.78	12.98	221.10	13.49	234.59	0.7947
	2533	16.71	111.43	12.55	13.49	154.18	9.56	163.74	0.7986
	2534	10.09	70.60	8.14	9.63	98.46	10.09	108.55	0.7892
	2559	21.24	79.54	12.71	5.51	119.00	13.30	132.30	0.7897
	2560	22.33	112.64	14.33	5.31	154.61	11.55	166.16	0.7831
	2561	26.39	112.39	13.79	4.42	156.99	13.79	170.78	0.7816
	2562	26.49	107.56	14.39	4.23	152.67	11.93	164.60	0.7726
	2563	32.16	107.05	13.60	5.42	158.23	15.36	173.59	0.7885

APPENDIX

TABLE 28 : Muscle water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to 30°C.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	CATIONS	Cl^-	CATIONS + Cl^-	H_2O CONTENT (kg/kg)
		(mmol/kg)							
03:00 h	3011	24.22	114.93	14.04	10.44	163.63	12.88	176.51	0.7983
	3012	25.78	113.61	13.17	12.07	164.63	10.20	174.83	0.8021
	3013	20.33	114.54	13.91	12.35	161.13	10.06	171.19	0.7914
	3014	19.03	105.54	15.49	10.22	150.28	9.87	160.15	0.7909
	3015	18.01	105.90	15.32	12.32	151.55	9.36	160.91	0.7866
	3032	21.31	112.90	14.30	10.61	159.12	8.74	167.86	0.7950
	3033	21.20	111.97	14.06	8.96	156.19	10.00	166.19	0.7941
	3034	19.04	107.35	-	13.74	-	8.84	-	0.7921
	3035	26.76	112.77	13.76	10.16	163.45	13.47	176.92	0.8020
	3059	28.49	90.94	12.78	3.81	136.02	13.49	149.51	0.7980
	3060	26.70	80.51	12.70	3.67	123.58	13.47	137.05	0.7744
	3061	26.62	100.76	13.69	3.51	144.58	13.43	158.01	0.7852
	3062	32.02	101.16	11.76	6.57	151.51	16.79	168.30	0.8052
	3063	27.92	95.88	13.08	3.40	140.28	12.62	152.90	0.8014
	3064	27.54	97.78	13.33	3.18	141.83	13.48	155.31	0.7953
09:00 h	3016	31.98	105.45	13.04	13.45	163.92	17.35	181.27	0.8119
	3017	20.47	111.68	15.01	14.89	162.05	10.62	172.67	0.7931
	3018	29.74	103.75	13.03	12.21	158.73	14.11	172.84	0.8148
	3019	17.69	110.45	14.81	7.14	150.09	8.50	158.59	0.7948
	3036	32.23	108.81	12.58	8.78	162.40	16.14	178.54	0.8164
	3037	28.53	113.23	13.71	10.53	166.00	11.46	177.46	0.7995
	3038	19.22	113.51	-	9.88	-	8.60	-	0.7876
	3039	28.21	103.44	12.58	11.07	155.30	14.03	169.33	0.8135
	3040	22.33	112.56	13.77	9.17	157.83	9.89	167.72	0.7937
	3065	26.55	102.41	12.66	3.06	144.68	13.69	158.37	0.7912
	3066	28.20	91.83	13.54	5.35	138.92	15.30	154.22	0.7829
	3067	24.10	105.37	14.70	5.38	149.55	12.20	161.75	0.7792
	3068	29.26	94.04	13.51	4.17	140.98	15.19	156.17	0.7844
	3069	25.46	102.27	13.88	5.17	146.78	13.85	160.63	0.7880
	3070	24.50	95.61	14.01	3.54	137.66	13.26	150.92	0.7639
15:00 h	301	23.47	118.54	14.30	11.16	167.47	11.34	178.81	0.7890
	302	20.42	117.65	14.89	8.39	161.35	9.20	170.55	0.7906
	303	18.44	112.15	16.42	10.44	157.45	9.24	166.69	0.7804
	304	19.48	105.22	15.06	8.02	147.78	10.11	157.89	0.7978
	305	25.37	96.06	13.79	8.14	143.36	15.06	158.42	0.8177
	3020	36.52	92.90	11.85	19.48	160.75	20.80	181.55	0.8110
	3021	22.40	116.59	14.89	10.89	164.77	11.27	176.04	0.7885
	3022	19.32	110.54	14.20	10.91	154.97	9.86	164.83	0.7923
	3023	72.36	58.06	8.56	14.87	153.85	-	-	0.8619
	3024	17.40	108.71	13.37	9.79	149.27	8.57	157.84	0.8029
	3025	22.94	96.06	12.39	13.96	145.35	11.64	156.99	0.8204
	3026	17.85	109.91	14.31	11.11	153.18	8.65	161.83	0.7989
	3027	19.43	109.55	14.44	15.75	159.17	10.21	169.38	0.7932
	3041	22.18	115.26	14.24	10.38	162.06	9.70	171.76	0.7845
	3042	31.31	102.88	12.38	10.87	157.44	15.35	172.79	0.8182
	3043	25.51	109.21	13.66	15.26	163.64	11.17	174.81	0.7999
	3044	21.12	113.59	14.07	11.42	160.20	9.27	169.47	0.7988
	3045	36.64	96.88	11.76	14.03	159.31	19.14	178.45	0.8185
	3046	22.10	111.09	14.57	4.35	152.11	11.31	163.42	0.7816
	3047	23.24	106.56	14.18	10.98	154.96	12.13	167.09	0.7869
	3048	49.07	80.00	10.03	4.90	144.00	34.25	178.25	0.8228
	3049	24.85	109.28	13.86	6.31	154.30	12.64	166.94	0.7977
	3050	25.77	99.03	14.65	3.91	143.36	12.32	155.68	0.7905
	3051	30.71	98.32	11.32	2.94	143.29	18.82	162.11	0.7804
	3052	49.45	73.56	9.73	10.09	142.83	32.08	174.91	0.8286
21:00 h	306	20.72	109.24	14.60	8.81	153.37	11.83	165.20	0.8048
	307	18.54	106.54	15.32	13.63	154.03	10.03	164.06	0.7902
	308	21.08	118.27	14.93	7.39	161.67	10.31	171.98	0.7890
	309	21.11	119.02	15.22	10.80	166.15	10.68	176.83	0.7873
	3010	23.91	116.20	13.60	11.09	164.80	12.61	177.41	0.8007
	3028	17.90	114.29	14.25	8.47	154.91	8.94	163.85	0.7986
	3029	20.50	112.72	13.67	8.68	155.57	10.13	165.70	0.7998
	3030	31.86	96.96	12.76	12.46	154.04	18.30	172.34	0.8118
	3031	20.51	118.59	13.89	12.08	165.07	9.47	174.54	0.7998
	3053	22.91	101.98	13.95	4.17	143.01	11.99	155.00	0.7866
	3054	25.93	92.50	11.79	9.33	139.55	12.80	152.35	0.8089
	3055	21.64	103.63	13.51	4.23	143.01	10.97	153.98	0.7906
	3056	32.41	80.86	11.95	3.44	128.66	17.47	146.13	0.7923
	3057	24.10	104.08	13.67	4.66	146.51	12.86	159.37	0.7913
	3058	27.09	101.26	13.06	7.60	149.01	14.29	163.30	0.7866

APPENDIX

TABLE 29 : Muscle water, electrolyte, total cation and total cation + Cl^- raw data for goldfish acclimated to a cycling temperature of $25^{o} \pm 5^{o}C$.

SAMPLING TIME	CODE NO	Na^+	K^+	Mg^{2+}	Ca^{2+}	TOTAL CATIONS	Cl^-	TOTAL CATIONS + Cl^-	H_2O CONTENT (Kg/kg)
		(mmol/kg)							
03:00 h	cy 11	24.00	91.65	11.01	3.37	130.03	13.36	143.39	0.8048
	cy 12	23.16	111.83	12.92	5.57	153.48	13.69	167.17	0.7921
	cy 13	20.94	61.76	12.82	3.74	99.26	8.25	107.51	0.7915
	cy 14	41.13	57.83	8.11	3.86	110.93	28.98	139.91	0.8382
	cy 15	16.69	100.23	13.90	4.65	135.47	6.77	142.24	0.7819
	cy 36	26.44	95.18	12.92	2.95	137.49	12.76	150.25	0.7776
	cy 37	29.55	103.27	14.03	2.01	148.86	15.84	164.70	0.7357
	cy 38	29.23	110.74	12.56	3.39	155.92	13.20	169.12	0.7858
	cy 39	33.03	109.57	13.38	4.23	160.21	13.00	173.21	0.7745
	cy 40	31.18	112.12	13.22	2.52	159.04	13.86	172.90	0.7821
	cy 59	21.65	99.75	12.03	3.98	137.41	13.10	150.51	0.7834
	cy 60	27.62	91.19	11.61	2.01	132.43	14.60	147.03	0.7848
	cy 61	24.00	110.32	12.55	2.93	149.80	11.77	161.57	0.7886
	cy 62	24.55	108.49	12.57	2.50	148.11	12.52	160.63	0.7869
	cy 63	25.46	96.40	12.21	3.69	137.76	14.48	152.24	0.7833
	cy 67	32.05	101.35	12.14	2.66	148.20	12.73	160.93	0.7870
	cy 68	35.36	94.13	12.11	2.47	144.07	13.87	157.94	0.7846
09:00 h	cy 16	21.29	106.63	12.31	2.37	142.60	13.72	156.32	0.8053
	cy 17	25.40	106.27	12.71	4.14	148.52	15.01	163.53	0.7857
	cy 18	23.33	92.34	12.80	6.75	135.22	13.62	148.84	0.7917
	cy 19	23.70	110.15	13.06	2.55	149.46	12.26	161.72	0.7799
	cy 20	29.67	92.46	13.01	3.60	138.74	15.97	154.71	0.7877
	cy 41	33.65	97.28	12.67	2.26	145.86	13.32	159.18	0.7717
	cy 42	34.35	96.89	12.77	1.99	146.00	12.77	158.77	0.7864
	cy 43	42.71	84.50	13.54	2.62	143.37	15.18	158.55	0.7564
	cy 44	26.93	104.62	12.24	1.80	145.59	14.66	160.25	0.7838
	cy 45	31.75	105.66	12.67	5.54	155.62	13.69	169.31	0.7888
	cy 54	30.41	95.26	11.26	2.26	139.19	16.97	156.16	0.7885
	cy 55	24.81	90.64	11.37	3.25	130.07	16.07	146.14	0.7879
	cy 56	34.75	86.38	11.56	3.82	136.51	16.75	153.26	0.7936
	cy 57	34.41	83.17	11.53	2.00	131.11	17.60	148.71	0.7770
	cy 58	32.03	90.04	11.78	2.52	136.37	15.23	151.60	0.7901
	cy 69	29.21	99.58	11.71	3.15	143.65	12.48	156.13	0.7886
	cy 70	35.00	92.30	11.33	3.98	142.61	13.01	155.62	0.7902
	cy 71	28.42	97.33	11.72	2.25	139.72	13.14	152.86	0.7883
15:00 h	cy 1	18.93	86.98	12.82	4.69	123.42	10.39	133.81	0.7860
	cy 2	29.67	103.88	11.87	6.09	151.51	19.87	171.38	0.8022
	cy 3	27.28	93.48	14.97	7.10	142.83	13.40	156.23	0.7374
	cy 4	22.19	64.16	12.90	9.46	108.69	9.45	118.14	0.7842
	cy 5	25.68	103.61	12.92	5.90	148.11	15.75	163.86	0.7892
	cy 21	24.23	99.63	12.15	4.19	140.20	13.96	154.16	0.7888
	cy 22	22.65	111.73	11.46	3.32	149.16	13.20	162.36	0.7838
	cy 23	24.19	115.83	11.70	6.58	158.30	17.44	175.74	0.7955
	cy 24	20.91	123.63	12.60	2.71	159.85	14.26	174.11	0.7894
	cy 25	23.44	110.30	11.45	1.64	146.83	13.33	160.16	0.7921
	cy 26	22.54	116.74	11.93	2.57	153.74	14.90	168.64	0.7912
	cy 27	20.16	119.69	12.21	2.05	154.11	13.21	167.32	0.7901
	cy 28	23.68	98.28	11.69	3.91	137.56	16.79	154.35	0.7898
	cy 29	23.44	120.29	14.13	2.93	160.79	12.56	173.35	0.7814
	cy 30	31.73	111.27	12.50	3.24	158.74	16.26	175.00	0.7849
	cy 46	26.02	109.64	13.05	6.64	155.35	13.69	169.04	0.7869
	cy 47	32.19	102.56	11.95	4.29	150.99	14.10	165.09	0.7879
	cy 48	30.80	107.57	11.85	3.94	154.16	15.46	169.62	0.7987
21:00 h	cy 6	16.42	92.80	13.89	7.38	130.49	8.42	138.91	0.7789
	cy 7	24.25	107.95	13.44	4.75	150.39	13.22	163.61	0.7905
	cy 8	18.28	65.74	13.56	2.49	100.07	6.07	106.14	0.7837
	cy 9	19.34	104.11	14.08	7.14	145.27	11.04	156.67	0.7988
	cy 10	22.84	104.56	12.46	3.13	142.99	14.42	157.41	0.7968
	cy 31	21.76	105.90	13.74	3.17	144.57	12.33	156.90	0.7640
	cy 32	32.24	106.91	12.24	4.29	155.68	15.34	171.02	0.7804
	cy 33	19.42	115.54	13.33	2.68	150.97	12.58	163.55	0.7725
	cy 34	25.10	102.89	13.13	4.30	145.42	13.64	159.06	0.7720
	cy 35	29.99	91.20	18.58	3.07	142.84	12.53	155.37	0.7269
	cy 49	27.10	99.76	13.54	2.62	143.02	14.24	157.26	0.7311
	cy 50	33.67	101.22	12.47	4.35	151.71	14.28	165.99	0.7805
	cy 51	32.91	93.59	12.72	2.34	141.56	15.64	157.20	0.7709
	cy 52	29.32	107.69	12.55	2.89	152.45	14.94	167.39	0.7865
	cy 53	27.33	104.38	12.14	2.38	146.23	16.39	162.62	0.7951
	cy 64	22.55	78.11	11.52	3.57	115.75	14.04	129.79	0.7780
	cy 65	34.00	79.43	11.07	5.69	130.19	12.08	142.27	0.7971
	cy 66	38.68	81.81	12.04	2.57	135.10	12.25	147.35	0.7854

APPENDIX

TABLE 30 : Ion : Hb ratio raw data for goldfish acclimated to 20°C.

SAMPLING TIME	CODE NO	Na^+/Hb	K^+/Hb	Mg^{2+}/Hb	Ca^{2+}/Hb	Cl^-/Hb
		(mmol/l packed cells/ mmol/l packed cells)				
03:00 h	203	5.20	33.13	2.88	0.082	20.02
	204	3.64	26.03	2.10	0.233	16.60
	2015	-	-	-	-	-
	2016	2.91	19.45	1.92	0.097	15.87
	2021	-	-	-	-	15.88
	2022	2.67	23.25	1.58	0.061	16.32
	2023	2.56	23.35	1.83	0.056	14.91
	2024	1.64	32.57	1.70	0.065	14.50
	2025	3.58	22.21	1.65	0.059	14.54
	2026	1.37	24.95	1.90	0.070	15.33
	2027	2.81	25.82	1.89	0.061	17.38
	2028	1.35	23.78	1.67	0.156	22.43
	2029	2.44	26.62	1.99	0.179	21.15
	2030	1.85	24.51	1.78	0.073	16.47
	2031	3.19	21.55	1.70	0.069	15.24
	2032	4.05	25.05	1.99	0.081	-
	2033	3.59	20.07	1.68	0.107	15.19
	2034	9.49	26.31	2.91	0.230	-
	2035	3.35	22.88	1.91	0.042	12.78
09:00 h	205	4.61	25.76	2.08	0.120	-
	206	-	-	-	-	-
	2017	-	- -	-	-	10.05
	2018	2.66	22.55	1.88	0.128	17.24
	2036	2.40	21.85	1.53	-	15.60
	2037	1.59	23.09	1.61	0.066	14.71
	2038	4.03	27.02	1.55	0.019	17.64
	2039	3.51	24.64	1.52	0.074	-
	2040	3.55	26.51	1.80	0.069	18.02
	2041	3.29	27.69	2.01	0.108	19.70
	2047	3.76	26.17	2.34	0.077	19.49
	2048	2.56	23.41	1.71	0.071	-
	2049	1.31	19.61	1.36	0.052	14.74
	2050	3.19	27.01	2.09	0.073	18.94
	2051	2.61	27.65	2.11	0.096	20.96
	2052	2.00	25.69	2.01	0.076	14.53
	2070	3.04	26.58	2.35	0.077	19.60
	2071	2.65	25.12	2.25	0.066	17.20
15:00 h	207	2.35	20.86	2.07	0.072	14.36
	208	4.74	30.98	2.54	0.417	21.68
	2011	2.93	22.25	1.48	0.175	16.18
	2012	-	-	-	-	-
	2019	-	-	-	-	-
	2020	-	-	1.92	0.094	-
	2053	1.94	23.94	1.48	0.082	19.92
	2054	2.60	23.13	2.02	0.052	17.30
	2055	2.03	21.99	1.39	0.057	17.52
	2056	1.75	28.04	1.93	0.073	20.26
	2057	1.80	22.84	1.60	0.060	17.08
	2063	2.49	23.66	1.91	0.071	19.69
	2064	1.86	23.31	1.82	0.074	-
	2065	3.32	24.70	1.94	0.065	20.53
	2066	2.11	21.73	1.74	0.053	16.82
	2067	2.62	22.81	1.92	0.062	18.92
	2072	3.17	25.86	2.41	0.067	17.15
	2073	3.79	28.40	2.51	0.073	-
21:00 h	201	3.70	24.97	1.79	0.116	-
	202	-	-	-	-	15.85
	209	1.27	21.41	1.58	0.080	-
	2010	1.97	23.82	1.87	0.072	18.61
	2013	2.53	25.65	2.16	0.099	-
	2014	2.18	25.03	2.03	0.094	20.61
	2042	2.79	19.21	1.65	0.052	15.64
	2043	1.52	23.68	1.84	0.079	16.12
	2044	2.46	23.34	1.52	0.072	16.85
	2045	3.37	25.27	1.99	0.072	18.92
	2046	2.30	20.50	1.53	0.057	14.83
	2058	3.42	24.95	2.18	0.071	21.10
	2059	2.71	25.26	1.95	0.077	20.65
	2060	2.55	23.54	1.66	0.083	18.71
	2061	2.82	22.20	1.79	0.072	16.68
	2062	3.68	23.52	1.89	0.083	21.12
	2068	4.16	32.16	3.17	0.091	20.92
	2069	2.96	26.09	2.40	0.077	16.88

APPENDIX

TABLE 31 : Ion : Hb ratio raw data for goldfish acclimated to 25°C.

SAMPLING TIME	CODE NO	Na^+/Hb	K^+/Hb	Mg^{2+}/Hb	Ca^{2+}/Hb	Cl^-/Hb
		(mmol/l packed cells/ mmol/l packed cells)				
0300 h	254	5.10	27.83	2.53	0.077	23.98
	255	12.48	32.20	2.05	0.178	35.12
	256	1.92	16.26	1.07	0.043	12.84
	2522	1.77	28.19	2.27	0.099	23.33
	2523	2.99	26.61	2.17	0.074	19.98
	2524	-	-	-	-	-
	2535	4.39	18.95	1.26	-	15.03
	2536	4.96	18.41	-	-	12.69
	2537	3.74	23.08	2.30	-	15.52
	2564	2.16	22.01	1.49	0.020	15.91
	2565	1.98	19.99	1.60	0.045	9.90
	2566	4.00	20.83	4.60	0.061	16.73
	2567	-	-	-	-	17.10
	2568	3.49	22.69	1.69	0.045	16.31
	2569	3.73	20.00	1.72	0.040	11.75
	2570	2.66	19.74	1.38	0.033	10.53
	2571	2.91	19.53	1.50	0.043	9.09
09:00 h	257	4.80	30.53	2.10	0.106	24.38
	258	-	-	-	-	-
	259	-	--	-	-	-
	2525	6.43	29.12	2.18	0.113	26.76
	2526	-	-	-	-	27.30
	2527	2.55	27.13	0.81	0.074	22.52
	2538	5.39	24.27	1.75	0.034	16.53
	2539	4.10	18.57	1.29	-	14.39
	2540	-	-	-	0.035	11.86
	2544	2.74	21.93	1.26	0.033	16.66
	2545	3.78	20.73	1.78	0.025	19.06
	2546	3.27	20.82	1.55	0.026	16.81
	2547	3.25	22.00	1.76	0.008	18.08
	2548	3.02	15.84	1.25	0.022	12.81
	2549	3.70	17.56	1.54	0.030	18.20
	2550	6.03	28.91	2.66	0.078	26.26
	2551	6.68	26.70	2.95	0.024	24.32
	2552	4.20	22.13	1.48	0.024	18.66
	2553	4.83	23.40	1.82	0.026	20.99
15:00 h	2510	2.61	25.47	1.56	0.043	23.58
	2511	5.38	30.99	2.45	0.105	-
	2512	2.47	23.52	2.00	-	20.10
	2516	3.02	22.70	1.64	-	-
	2517	3.82	27.71	2.25	0.122	24.12
	2518	-	-	-	-	-
	2528	3.78	27.22	2.28	0.049	22.72
	2529	4.50	28.16	2.42	0.097	24.20
	2530	1.81	7.01	0.56	-	13.63
	2531	1.60	20.85	1.39	-	14.20
	2541	3.78	21.51	1.42	-	14.80
	2542	4.61	21.25	1.58	-	17.28
	2543	3.79	21.54	1.60	-	16.88
	2554	1.96	22.00	1.36	0.017	19.50
	2555	3.35	23.24	1.57	0.035	21.40
	2556	3.05	20.63	1.54	0.023	17.37
	2557	3.20	20.80	1.65	0.005	18.20
	2558	1.49	26.22	1.51	0.019	19.84
21:00 h	251	2.70	25.26	1.99	0.119	23.77
	252	-	-	-	-	-
	253	7.00	29.51	2.26	0.085	26.82
	2513	2.56	25.06	2.01	0.093	19.24
	2514	4.15	25.47	1.99	0.071	20.66
	2515	3.82	24.99	1.71	0.047	21.96
	2519	4.07	28.42	2.16	0.108	27.07
	2520	4.50	26.47	2.36	0.074	25.14
	2521	2.67	27.21	2.30	0.083	-
	2532	5.42	22.29	1.42	-	18.58
	2533	6.17	24.52	1.77	0.060	15.80
	2534	4.58	23.18	1.55	-	17.11
	2559	-	-	-	-	-
	2560	1.54	22.58	1.45	0.018	16.43
	2561	2.46	25.57	1.61	0.023	16.79
	2562	1.91	24.42	1.76	0.027	17.80
	2563	2.27	23.70	1.60	0.016	18.42

APPENDIX

TABLE 32 : Ion : Hb ratio raw data for goldfish acclimated to 30°C.

SAMPLING TIME	CODE NO	Na^{+}/Hb	K^{+}/Hb	Mg^{2+}/Hb	Ca^{2+}/Hb	Cl^{-}/Hb
		(mmol/l packed cells/mmol/l packed cells)				
03:00 h	3011	3.81	19.58	1.27	0.026	16.13
	3012	1.80	18.28	1.51	0.013	13.98
	3013	3.47	21.14	1.49	0.032	17.17
	3014	2.80	17.81	1.06	0.018	13.19
	3015	3.64	19.71	1.21	-	15.94
	3032	4.57	23.02	1.55	-	18.22
	3033	5.60	21.22	1.54	0.005	17.11
	3034	4.46	22.82	1.35	-	18.10
	3035	4.95	23.32	1.51	0.020	17.15
	3059	4.51	26.73	1.65	0.017	17.74
	3060	3.36	25.34	1.71	0.014	16.42
	3061	3.39	25.45	1.55	0.016	17.44
	3062	2.93	23.89	1.67	0.026	16.15
	3063	3.77	26.19	1.35	0.024	19.41
	3064	2.27	25.26	1.44	0.024	19.17
09:00 h	3016	-	-	-	-	24.44
	3017	4.09	20.10	1.34	0.002	16.83
	3018	3.88	23.21	1.62	0.029	19.50
	3019	3.23	22.16	1.28	0.029	16.95
	3036	8.58	30.22	2.31	0.079	29.58
	3037	5.12	24.64	1.86	0.016	17.31
	3038	5.34	23.45	1.46	-	18.75
	3039	4.46	19.76	1.30	0.017	16.51
	3040	5.02	20.16	1.56	0.005	16.94
	3065	3.30	26.53	1.68	0.023	19.56
	3066	2.98	25.29	1.51	0.023	19.28
	3067	2.96	26.08	1.38	0.022	19.81
	3068	1.93	29.65	1.58	0.028	22.33
	3069	3.04	28.70	1.91	0.026	22.25
	3070	3.05	27.07	1.94	0.026	19.46
15:00 h	301	2.35	19.76	1.18	0.012	16.94
	302	6.87	31.97	1.68	0.178	20.02
	303	4.21	19.96	1.27	0.027	14.81
	304	3.48	20.72	1.42	-	14.46
	305	2.98	20.82	1.34	-	15.72
	3020	3.45	25.33	2.16	0.041	22.47
	3021	4.61	21.08	1.28	0.016	17.76
	3022	5.21	22.43	1.71	0.011	17.57
	3023	4.13	25.33	2.01	0.019	22.32
	3024	2.94	20.59	1.38	0.014	20.25
	3025	4.05	22.21	2.42	0.086	19.54
	3026	5.36	27.46	2.17	0.112	20.81
	3027	1.93	22.24	1.52	0.011	15.94
	3041	5.92	21.00	1.31	0.072	19.24
	3042	4.78	24.38	1.82	0.008	18.74
	3043	4.45	19.98	1.64	0.067	17.04
	3044	5.38	23.38	1.78	0.133	20.08
	3045	6.58	25.20	1.91	0.014	20.74
	3046	4.41	23.39	1.42	0.016	17.43
	3047	3.04	24.71	1.38	0.007	19.27
	3048	3.95	24.58	1.31	0.003	19.40
	3049	3.04	25.41	1.43	0.008	21.21
	3050	4.63	22.91	1.53	0.009	18.75
	3051	3.57	11.87	0.75	0.008	10.51
	3052	5.69	46.75	3.04	0.037	31.34
21:00 h	306	3.47	20.28	1.36	0.014	17.53
	307	1.95	18.55	1.09	0.011	14.94
	308	4.48	21.11	1.52	0.004	17.19
	309	4.08	19.12	1.28	0.047	13.21
	3010	3.43	20.98	1.42	0.014	16.95
	3028	9.17	33.64	2.99	0.112	27.12
	3029	5.17	22.83	1.90	0.036	-
	3030	4.71	24.77	1.66	0.026	21.03
	3031	5.47	23.11	1.81	0.056	-
	3053	3.36	26.73	1.71	0.016	24.31
	3054	3.58	21.70	1.49	0.016	15.95
	3055	2.70	24.68	1.77	0.019	17.32
	3056	2.73	26.25	1.71	0.016	18.63
	3057	2.72	21.93	1.42	0.015	19.06
	3058	3.30	25.11	1.67	0.016	16.16

APPENDIX

TABLE 33 : Ion : Hb ratio raw data for goldfish acclimated to a cycling temperature of $25^{\circ} \pm 5^{\circ}C$.

SAMPLING TIME	CODE NO	Na^+/Hb	K^+/Hb	Mg^{2+}/Hb	Ca^{2+}/Hb	Cl^-/Hb
		(mmol/l packed cells/ mmol/l packed cells)				
03:00 h	cy 11	1.68	18.28	1.26	0.011	12.65
	cy 12	-	22.08	1.25	0.013	15.55
	cy 13	2.50	23.62	1.68	0.035	16.38
	cy 14	1.20	22.28	1.52	0.025	14.27
	cy 15	1.72	22.15	1.83	0.017	13.18
	cy 36	2.18	21.67	1.73	0.015	-
	cy 37	1.61	25.65	1.82	0.025	18.59
	cy 38	1.91	25.12	1.73	0.024	17.41
	cy 39	2.04	24.49	1.95	0.022	15.84
	cy 40	1.97	24.72	1.94	0.018	16.68
	cy 59	1.33	25.41	1.44	0.025	18.85
	cy 60	1.51	27.30	1.53	0.028	19.16
	cy 61	-	-	-	-	18.20
	cy 62	1.89	25.56	1.88	0.029	16.75
	cy 63	2.18	28.65	2.10	0.032	18.53
	cy 67	3.09	23.00	1.79	0.033	14.84
	cy 68	2.04	21.70	1.62	0.038	15.68
09:00 h	cy 16	1.46	21.60	1.22	0.016	15.78
	cy 17	2.36	22.05	1.65	0.016	14.55
	cy 18	2.10	23.10	1.58	0.016	14.40
	cy 19	1.72	23.44	1.70	0.017	15.77
	cy 20	1.64	20.03	1.43	0.014	13.36
	cy 41	1.94	23.34	1.72	0.022	16.67
	cy 42	3.01	22.89	1.60	0.023	18.55
	cy 43	1.91	23.75	1.53	0.025	15.84
	cy 44	3.29	22.56	1.79	0.020	14.34
	cy 45	4.34	22.76	1.76	0.021	17.50
	cy 54	3.13	25.91	1.88	0.026	21.12
	cy 55	3.38	24.18	1.95	0.027	17.51
	cy 56	4.55	25.34	1.95	0.028	-
	cy 57	3.70	24.45	1.83	0.025	17.49
	cy 58	3.20	25.28	1.51	0.025	17.99
	cy 69	3.76	21.54	1.66	0.025	16.32
	cy 70	5.06	21.99	1.75	0.031	17.33
	cy 71	3.15	22.25	1.79	0.027	15.16
15:00 h	cy 1	2.25	23.80	1.36	0.012	16.80
	cy 2	2.87	22.98	1.66	0.012	15.11
	cy 3	2.30	23.54	1.58	0.009	16.31
	cy 4	3.17	25.36	1.57	0.134	19.17
	cy 5	3.74	23.60	0.63	0.016	15.76
	cy 21	2.75	24.05	1.66	0.017	18.21
	cy 22	3.98	25.53	2.04	0.018	17.97
	cy 23	2.88	24.05	1.53	0.021	19.98
	cy 24	2.32	28.96	2.04	0.017	21.25
	cy 25	3.89	22.83	1.54	0.021	17.96
	cy 26	3.46	24.64	2.00	0.014	19.55
	cy 27	4.87	25.47	2.00	0.016	20.81
	cy 28	3.46	24.48	1.62	0.019	18.69
	cy 29	4.70	25.84	1.72	0.030	19.79
	cy 30	3.53	26.63	2.10	0.013	19.22
	cy 46	3.40	22.97	1.77	0.018	17.42
	cy 47	4.02	22.43	1.70	0.020	16.53
	cy 48	3.74	24.70	2.00	0.023	18.37
21:00 h	cy 6	2.56	23.64	0.66	0.035	16.65
	cy 7	1.38	25.30	1.75	0.014	16.74
	cy 8	1.35	23.06	1.72	0.092	15.58
	cy 9	1.18	20.82	1.68	0.013	13.81
	cy 10	1.02	29.71	2.15	0.017	19.61
	cy 31	2.00	23.11	1.92	0.011	16.07
	cy 32	2.18	25.28	1.97	0.018	19.89
	cy 33	2.43	22.10	1.65	0.015	15.39
	cy 34	3.37	25.78	1.85	0.021	18.81
	cy 35	2.77	25.46	1.81	0.020	18.19
	cy 49	3.42	22.95	1.76	0.021	16.35
	cy 50	4.77	23.57	1.88	0.026	18.24
	cy 51	4.68	22.07	1.83	0.027	17.50
	cy 52	3.92	22.49	1.77	0.023	17.39
	cy 53	3.21	24.80	1.68	0.076	21.61
	cy 64	2.45	22.19	1.63	0.037	13.05
	cy 65	3.54	21.85	1.83	0.025	14.08
	cy 66	2.54	20.25	1.36	0.032	14.24

APPENDIX IV

Experimental animals were obtained from a number of sources. This was done in order to obtain specimens covering a sufficient weight range to permit evaluation of possible weight effects upon thermal responses (Houston and Smeda, 1979). Regression analyses were performed on hemoglobin content and packed cell volume values with respect to weight (Appendix Tables 34 and 35). Since there was little evidence of weight-specific variation, the trends observed (see Figure 12A) could not be linked to source of origin. Further evidence for this conclusion was obtained from the results of analyses of variance (ANOVA) carried out on packed cell volume data (Appendix Table 36). Significant differences based on source of origin were rarely encountered. When they did, they were not consistent with respect to specimen source, nor did they present a coherent picture in relation to time or temperature of occurrence. A direct relationship between the trends seen under any one thermal regime (see Figure 12A) and the results of ANOVA testing could not be detected. Thus, it is concluded that specimen source did not represent a significant variable in the study.

APPENDIX

TABLE 34 : Regression analysis results of hematological parameters versus weight at the sampling times of the four acclimation temperature regimes.

HEMATOLOGICAL PARAMETER.	TEMP.	TIME	REGRESSION	N	A	B	0 DEGREE COEFFIC.	1 DEGREE COEFFIC.	COEFFIC. OF CORREL.	SIGNIF. LEVEL	STANDARD ERROR
Hemoglobin Content (gm/100 ml)	20°C	15:00	Linear	17			5.956	0.081	0.5215	P<0.05	1.3873
		21:00	Linear	18			7.404	0.018	0.1061	N.S.	1.4072
		03:00	Linear	18			4.334	0.044	0.7777	P<0.01	0.9247
		09:00	Linear	18			6.031	0.030	0.1540	N.S.	1.9381
	25°C	15:00	Geometric	17	4.891	0.13205			0.4614	N.S.	0.1897
		21:00	Geometric	17	3.704	0.21464			0.7472	P<0.01	0.7472
		03:00	Geometric	17	3.278	0.26781			0.6615	P<0.01	0.2074
		09:00	Linear	19			5.371	0.050	0.6253	P<0.01	1.0029
	30°C	15:00	Geometric	24	9.016	-0.03553			0.0993	N.S.	0.2448
		21:00	Linear	14			7.479	-0.013	0.0822	N.S.	1.6333
		03:00	Geometric	14	19.461	-0.23514			0.5112	N.S.	0.1440
		09:00	Linear	15			8.633	-0.026	0.1400	N.S.	2.4720
	25°± 5°C.	15:00	Linear	17			8.494	-0.007	0.1112	N.S.	0.8468
		21:00	Geometric	17	5.845	0.10602			0.2091	N.S.	0.1054
	25 ± 5 C.	03:00	Geometric	16	8.494	-0.01475			0.0411	N.S.	0.0850
		09:00	Linear	16			9.017	-0.007	0.0747	N.S.	0.8564
Packed Cell Volume (%)	20°C	15:00	Linear	17			0.489	0.003	0.4760	N.S.	0.0529
		21:00	Linear	17			0.558	0.0007	0.1619	N.S.	0.0465
		03:00	Linear	18			0.454	0.0013	0.5835	P<0.05	0.0482
		09:00	Geometric	17	0.6628	-0.06929			0.2224	N.S.	0.1300
	25°C	15:00	Linear	16			0.587	-0.0004	0.2277	N.S.	0.0326
		21:00	Geometric	15	0.5501	-0.0036			0.0975	N.S.	0.0490
		03:00	Geometric	16	0.5211	0.02139			0.1913	N.S.	0.0774
		09:00	Geometric	18	0.5681	-0.02426			0.1690	N.S.	0.0828
	30°C	15:00	Linear	23			0.575	-0.0004	0.2949	N.S.	0.0583
		21:00	Linear	14			0.504	0.00019	0.0582	N.S.	0.0348
		03:00	Geometric	14	0.7804	-0.09135			0.3863	N.S.	0.0795
		09:00	Linear	13			0.625	-0.0019	0.5502	N.S.	0.0401
	25°± 5°C.	15:00	Linear	17			0.618	-0.0013	0.4796	N.S.	0.0315
		21:00	Linear	17			0.581	-0.0003	0.0651	N.S.	0.0339
		03:00	Geometric	16	0.5535	0.50859			0.0289	N.S.	0.0687
		09:00	Geometric	17	0.6771	-0.04847			0.2240	N.S.	0.0592

APPENDIX

TABLE 35: Results of Regression Analysis Involving Hematological Parameters and Weight Values for Totals Obtained at Each Acclimation Temperature.

HEMATOLOGICAL PARAMETER	TEMPERATURE	REGRESSION	N	A	B	0 DEGREE COEFFICIENT	1 DEGREE COEFFICIENT	COEFFICIENT OF CORRELATION	SIGNIFICANCE LEVEL	STANDARD ERROR
Hemoglobin Content (gm/100 ml)	20°C	Linear	71			6.484	0.028	0.2674	$P<0.05$	1.6299
	25°C	Geometric	70	3.6786	0.21429			0.6084	$P<0.01$	0.1802
	30°C	Geometric	67	8.9784	-0.04432			0.0813	N.S.	0.2625
	$25^\circ \pm 5$ C	Linear	66			8.516	-0.004	0.0435	N.S.	0.8354
Packed Cell Volume (%)	20°C	Linear	69			0.528	0.0005	0.1308	N.S.	0.0624
	25°C	Geometric	65	0.5560	-0.00074			0.0064	N.S.	0.0751
	30°C	Geometric	64	0.6630	-0.05421			0.2729	$P<0.05$	0.0925
	$25^\circ \pm 5^\circ$C.	Linear	67			0.599	-0.0008	0.2245	N.S.	0.0334

APPENDIX

TABLE 36 : Results of analysis of variance upon packed cell volume data based on source of origin

TEMP. (°C)	SAMPLING TIME (hr)	SOURCE COMPARISON MADE	SIGNIFICANT DIFFERENCE
20	03:00	H*(4)** Vs C(8)	N.S.
		H(4) Vs W(6)	N.S.
		C(8) Vs W(6)	$P < 0.05$
	09:00	H(3) Vs M(14)	N.S.
	15:00	H(5) Vs M(12)	N.S.
	21:00	H(6) Vs M(11)	N.S.
25	03:00	H(6) Vs C(2)	N.S.
		H(6) Vs W(8)	N.S.
		C(2) Vs W(8)	N.S.
	09:00	H(6) Vs C(6)	$P < 0.05$
		H(6) Vs W(5)	N.S.
		C(6) Vs W(5)	N.S.
	15:00	H(7) Vs C(4)	N.S.
		H(7) Vs W(5)	N.S.
		C(4) Vs W(5)	N.S.
	21:00	H(7) Vs C(4)	N.S.
		H(7) Vs W(4)	N.S.
		C(4) Vs W(4)	N.S.
30	03:00	C(9) Vs W(5)	N.S.
	09:00	C(8) Vs W(5)	N.S.
	15:00	C(16) Vs W(7)	N.S.
	21:00	C(9) Vs W(5)	N.S.
25± 5	03:00	C(1) Vs W(3)	N.S.
		C(1) Vs M(12)	N.S.
		W(3) Vs M(12)	N.S.
	09:00	C(1) Vs W(3)	N.S.
		C(1) Vs M(12)	N.S.
		W(3) Vs M(12)	N.S.
	15:00	C(5) Vs W(5)	$P < 0.01$
		C(5) Vs M(7)	N.S.
		W(5) Vs M(7)	$P < 0.01$
	21:00	C(2) Vs W(2)	N.S.
		C(2) Vs M(13)	N.S.
		W(2) Vs M(13)	$P < 0.05$

* H = Hartz Mountain
C = Carolina Biological
W = Wild
M = Tropic Aquaria

** = Sample size